1967

# Serological predictions of genotypic relationships among oat varieties using antigens from different plant parts

Rex Lanel Smith
*Iowa State University*

# SEROLOGICAL PREDICTIONS OF GENOTYPIC RELATIONSHIPS AMONG OAT VARIETIES USING ANTIGENS FROM DIFFERENT PLANT PARTS

by

Rex Lanel Smith

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Crop Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1967

# TABLE OF CONTENTS

# INTRODUCTION

The goal of plant breeding is to produce superior genotypes that will result in improved plant performance. One major technique of achieving this goal is to use extensive hybridization programs to bring about genetic recombination. Since it is not practical to make all possible hybrid combinations among a series of parents, and since few crosses produce superior genotypes, breeders continually search for techniques to predict which crosses will be the most lucrative. At present the only reliable method for selecting the best crosses in self pollinated crops is to make crosses and study the magnitude of genetic variability within each cross. In cross pollinated crops, predictions of the best hybrid combinations are made by studying the vigor of single crosses. In either case, the production and assay of these crosses require a large expenditure of time and resources, and in many cases costs may be prohibitive.

In general, the most vigorous single crosses of corn and the small grain crosses with the greatest magnitude of segregation are from parents that are most genetically diverse. Therefore, an efficient method for assaying genetic relationship among genotypes would be valuable for predicting the best crosses to make. This would allow the breeder to survey many genotypes as potential parents.

It would be Utopian if the plant breeder could determine genetic relationship by direct chemical analysis of the genetic material, deoxyribonucleic acid (DNA). At present this is not possible. Molecular genetics research has shown, however, that the DNA controls protein synthesis through a series of reactions involving messenger RNA, transfer RNA and ribosomes. Therefore, the diversity of the protein moieties of enzymes responsible for biosynthesis in different genotypes might provide a crude index of genetic diversity.

There are many methods of characterizing individual proteins, but these are of limited value for comparing the complex mixture of proteins of one genotype with that of another. Serology is perhaps the best method for comparing complex protein mixtures. It is the study of antigen-antibody reactions. An antigen is a large complex molecule which when injected into an animal stimulates the production of specific antibodies. Antibodies can recognize and react with the same or similar antigens that caused their production. Proteins are the most important plant antigens. Although antigen-antibody reactions are basic mechanisms in animals, they also occur in vitro.

The characteristic that makes serology useful for comparing proteins from different genotypes is its specificity, i.e., with few exceptions, antibodies only react with antigens similar to those that stimulated their production. Antigen

(protein) specificity depends upon the chemical composition and the spatial configuration of the molecule. Very small differences in antigen composition can be detected by these reactions (Landsteiner, 1946).

Although serology has been used since the turn of the century, its usefulness to study plant and animal relationships, in general, has been limited to distinguishing among genera or species. Kleese and Frey (1964) showed that the serological technique of Boyden and DeFalco (1943) could predict genetic relationships among varieties of oats. However, Kleese (1962) could not use the technique to assay genetic relationships of corn.

My objectives herein were (a) to attempt to corroborate the conclusions of Kleese and Frey (1964), namely, that the quantitative serological method could assay genetic relationships among oat genotypes, (b) to compare the values of antigens from different plant parts for predicting genetic relationships of oat varieties, and (c) to study the qualitative serological differences among antigens from the different plant parts.

# REVIEW OF LITERATURE

## Serology in Plant Systematics

Serology has been used in the study of animal and plant relationships since 1900. The precipitin reaction, which is the most important reaction in plant sero-systematics, was first reported by Kraus (1897). According to Boyden (1954), Bordet (1899) was the first to describe heterologous precipitin reactions between anti-chicken serum and chicken and pigeon serum. Subsequently, other workers reported heterologous reactions (Myers, 1900; Uhlenhuth, 1900; Nuttall, 1901) that established the basis of sero-systematics, namely, that closely related heterologous antigens can react with antisera, but with less intensity than the homologous antigens. Nuttall (1904) published a classic work summarizing data from 16,000 tests performed on 900 samples of blood.

Use of serology to study plant relationships was suggested by Kowarski (1901). His sera, from rabbits injected with heat resistant wheat albumose, reacted strongly with wheat albumose, but weakly or not at all with the albumoses of rye, barley, oats and peas. An extensive review of serology in plant systematics was made by Chester (1937).

A group of studies on sero-systematics known as the Konigsberg series was described by Chester (1937). This work, which began in 1913, was aimed at extending sero-systematics

to cover the entire plant kingdom. The work, done under the direction of Mez, was summarized in a genealogical tree or "Stammbaum." Because the Konigsburg's "Stammbaum" did not agree completely with the accepted concepts of taxonomy it was strongly criticized. According to Chester (1937), Gilg and Schurhoff (1927) began studies in Berlin in 1926 to re-test the correctness of the "Stammbaum" and to evaluate methods used by the Konigsberg group. They concluded that serology was satisfactory for identifying plant antigens, but that sero-diagnostics was not useful to study botanical relationships.

Chester (1937) indicated that the contradictory results were due in large part to the differences in techniques employed by the two groups. A major difference was the extraction method used. The Konigsberg group used extensive pre-extraction methods to remove substances that caused non-specific reactions and the Berlin group did not because they thought this treatment denatured and altered the antigens. As a result the tests of the latter group were confounded by non-specific reactions.

According to Chester (1937), additional research refuted the data of the Berlin group. However, the controversy produced a general lack of confidence in serology.

An accurate, rapid, quantitative method of measuring the intensity of precipitin reactions was reported by Boyden and

DeFalco (1943). It utilizes a photoelectric instrument developed by Libby (1938) to measure turbidity which is proportional to the precipitin reaction. This method measures the entire titration curve for a given antiserum titrated with progressive dilutions of antigens. According to Boyden et al. (1947), the entire curve from excess antigen to excess antibody should be measured. They considered the methods of ring or flocculation titer, point of optimal proportions, point of maximum precipitation, and neutralization point gave biased data, because titrations with a single antiserum and a single antigen generally have different optima points on the titration curves. The entire titration curve can be measured quantitatively using the micro-Kjeldahl method, but the photoelectric method requires less antisera, antigen and time.

Hammond (1952) used the serological technique of Boyden and DeFalco (1943) to study the relationships of Ranunculaceae. His sero-systematic data corroborated the plant relationships outlined by taxonomists who used morphological and cytological data. Johnson (1954) studying Magnoliaceae, Baum (1954) studying Cucurbitaceae and Hammond (1955) studying Solanaceae concluded that serological data was a valuable supplement to morphological data for classifying these families. Fairbrothers and Johnson (1959) said that even though phytoserology is complex, it is a valuable tool for taxonomic study of forage grasses.

Kloz et al. (1959) studied the serological specificity of proteins from various parts of plants in the family Viciaceae. Reserve proteins of the cotyledon were more species specific than leaf proteins and subcotyledon proteins from different organs of the same plant than among proteins of the same organ from different species.

Gell et al. (1959) objected to methods which quantitatively measure total turbidity or total amount of precipitate as a measurement of relationship because it is impossible to distinguish between qualitative antigen differences and quantitative differences of common antigens. They used the immunoelectrophoretic technique in their taxonomic study with the genus Solanum. This technique separates the antigens by differential rates of migration through a gel under the influence of an electric field into bands that can be identified. The relationships among species Gell et al. (1959) observed were generally similar to those already postulated by morphologic and cytogenetic studies. Hall (1959), using immunoelectrophoresis, found that wheat and rye have some proteins in common and some not in common. The rye-wheat hybrid had all the proteins of wheat, all but one of rye, but none specific to itself.

## Comparative Serology on the Species Level

Although Zade (1914) reported that varieties could be distinguished serologically, it was generally accepted that the genera or species was the limit of resolution with serology. Nelson and Birkeland (1929) were the first to serologically measure genetic relationships within a species by ranking five wheat varieties. With refined serological techniques such as carefully purifying seed proteins and adsorbing antisera they showed that the serological and genetic rankings of the wheat varieties were parallel.

Urano (1955) found that two lines of maize from the same $S_{10}$ plant gave stronger serological reaction than lines from the same $S_3$ or $S_5$ plant. Yamasaki et al. (1957) reported similar results and in addition they used serology to predict hybrid vigor. They used three diverse groups of corn races, each with a different number of chromosome knobs. From crosses between inbreds from these groups they calculated indices of heterosis for grain yield, and indices of phylogenetic relationship were obtained from numbers of hetero-pairs of chromosome knobs. The intensity of serological reactions showed a high negative correlation with the heterosis indices and a high positive correlation with the phylogenetic indices. They concluded that $F_1$ hybrid vigor could be predicted by the serological index, also that hybrid vigor predicted by serological means was dependent upon the degree of heterozygosity.

Urano (1959) later reported a high negative correlation between serological indices of the parent lines and heterosis of $F_1$ hybrids. The serological relationship of the single cross to either parent was greater than the relationship of one parent to the other; the serological relationship of the backcross to the recurrent parent was greater than the single cross to the same parent. He concluded that serological data may be used as indices of phylogenetic relationship and to predict the degree of heterotic vigor in $F_1$'s. The maize pollen extracts used by Urano (1959) consisted mainly of albumin and $\alpha$-globulins, but the $\beta$- and $\gamma$-globulins were also present. The phylogenetic specificity resided with the albumin fraction, and value of antigens in studying phylogenetic relationships were in the order, non-heated pollen extracts, non-heated seed extracts, heated pollen extracts.

Davidson and Thompson (1956) studied the serological relationships of six lines of maize with known genealogies. Although the serological and genealogical rankings of five dent lines were in agreement, they concluded that serology was an inadequate criterion of relationship because of the inconsistent ranking of the one pop corn line.

Kleese and Frey (1964) studied several serological methods to determine which would best predict the genetic relationships of seven oat varieties and four corn inbreds. The serological data was correlated with genetic relationships based

on the within-cross variance components of oat lines and the relative vigor of the single crosses of corn inbreds. Immunodiffusion in agar and on cellulose acetate and immunoelectrophoresis were not useful techniques. The serological relationships of the oat varieties obtained by the quantitative method of Boyden and DeFalco (1943), agreed well with the relationships based on the grain yield variance components. Serological relationships of these same varieties calculated from reciprocal comparisons did not agree with the genetic indices of relationship. No confidence was placed in the latter comparisons because the antisera were drawn over an extended period which may have caused a loss of specificity. The serological relationships of the corn inbreds did not agree with the vigor indices of their hybrids.

Exposito et al. (1966), using the gel diffusion serological technique, were able to identify nine varieties of alfalfa seed.

## Relationship of Protein to Genotype

Kraus (1897) recognized that antigens of the precipitin reaction were proteinaceous, and generally, the most important antigens in plant and animal sero-systematics consist mainly of protein. To use serology to study phylogenetic relationships, a connection must exist between the genotype of a plant

and the proteins it contains. In the early work, serology was justified on the same empirical basis as morphology, i.e., organisms which had characteristics in common were judged to be more closely related than those that did not.

Mendel's work established the gene as a distinct unit which controlled an organism's characteristics. According to Gardner (1960), T. Boveri and W. S. Sutton established that genes are located on the chromosomes. Although chromosomes were known to consist of nucleic acids and protein, as late as 1939 Sturtevant and Beadle (1939) stated that the chemistry of the gene was unknown. They suggested that genes were protein and thus might act directly as enzymes or produce enzymes as direct products.

The first evidence that genes exerted control through enzymatic action was presented by Garrod (1909) (according to Srb et al., 1965). His work on the heritable human disease, alcaptonuria, was considered by Srb et al. (1965) to be the beginning of biochemical genetics. Beadle and Tatum (1941) studied X-ray induced nutritional mutants of Neurospora crassa and found that single gene mutations blocked certain steps in the production of essential vitamins. This work led to the "one gene-one enzyme" theory.

Horowitz and Leupold (1951) reviewed work supporting the "one gene-one enzyme" theory and concluded that the great majority of nutritional mutants in Neurospora require single

substances as growth factors. This theory is supported by work from the bacteria, Escherichia coli, (Umbarger and Mueller, 1951). Schwartz (1960), working with maize, and Langridge and Brock (1961), working with tomatoes, found mutants caused by blocked metabolism due to the absence of a single enzyme.

Ingram (1957) found that sickle cell and normal hemoglobins differed because a glutamic acid was replaced by a valine in the 300-amino-acid molecule of the $\beta$ chain. This was the first demonstration that a single gene mutation changed one amino acid in a polypeptide chain. Since the $\alpha$ and $\beta$ chains of hemoglobin are inherited via different loci the "one gene-one enzyme" theory was renamed "one gene-one polypeptide." Schroeder (1963) has reviewed other abnormal hemoglobins caused by amino acid substitutions due to single gene mutations.

Even though these studies have demonstrated that the genes control the biochemical reactions in a cell, they have given no insight into the specific mechanisms involved.

The establishment of DNA as the genetic material began when Griffith (1928) observed the transformation of an avirulent strain of pneumococci to a virulent form. Mice injected with a live, rough, avirulent strain of pneumococci along with heat-killed, smooth, virulent organisms often succumbed from pneumonia. Dawson and Sia (1931) continued this work by performing in vitro transformation of pneumococci. Transformation was solved when Avery et al. (1944) isolated a highly purified,

biologically active fraction from heat-killed type III pneumococci capable of inducing the transformation of unencapsualted R type variants to fully encapsulated cells of the same type as the inducing material. Extensive analyses, run on this fraction showed that it was mainly, if not solely, highly polymerized DNA. It did not contain demonstrable protein.

Another demonstration that DNA, not protein, was the genetic material was presented by Stadler and Uber (1942) using maize. Pollen was irradiated with different wave lengths of ultra-violet light and the mutagenic effects were observed in the progeny. The aberrations plotted against the wave length followed closely the absorption spectra of DNA.

Hershey and Chase (1952) labeled the protein of T2 with $S^{35}$ and its DNA with $P^{32}$. The phage progeny contained 30% of $P^{32}$ and only 1% of $S^{35}$ showing that the DNA was passed to the progeny.

Adding to the proof, Fraenkel-Conrat and Singer (1957) degraded tobacco mosaic virus (TMV) into protein and nucleic acid fractions, and the viruses were reconstituted with the protein of one strain being united with the nucleic acid of another. The disease invoked by the reconstituted virus resembled that of the strain contributing the nucleic acids, and its serological reactions were similar to those of the strain supplying the protein.

Watson and Crick (1953a and 1953b) proposed that DNA, for

which they made a molecular model based on X-ray diffraction studies by Wilkins et al. (1953), was the hereditary material. The model consisted of two helical chains coiled around one axis with nucleotide bases on the inside. The two chains are held together by hydrogen bonding of the bases, a purine in one chain binding to a pyrimidine in the other. There was no restriction in the order of the bases in a single chain, but the order of one chain fixed the order of the other automatically. Watson and Crick (1953c) proposed that the specific pairing provided a copying mechanism in which each chain served as a template for the other, and the order of bases in a chain gave the molecule its specificity.

Zamecnik (1959) presented a review of protein synthesis from a biochemist's viewpoint. Since the peptide bond was endergonic (requiring a source of free energy), biochemists postulated that a high energy intermediate compound was involved in protein synthesis. His review covers amino acid activation, the coupling of soluble RNA and its role in determining the amino acid sequence in polypeptide synthesis.

The existance of an RNA complementary to DNA was reported by Spiegelman et al. (1961), who found natural DNA-RNA hybrids in E. coli cells infected with T2 phage. Hayashi and Spiegelman (1961) reported that RNA from uninfected E. coli had a base ratio analogous to its homologous DNA. This RNA was metabolically unstable and had the ability to hybridize with its

homologous DNA. Nirenberg and Matthaei (1961) found that protein synthesis in a cell-free system was dependent upon the addition of heat-stable template RNA, and that soluble RNA could not replace template RNA. Bremer and Konrad (1964) synthesized RNA in vitro, using purified DNA-dependent RNA polymerase.

According to a review by Watson (1963), the ribosomes are the sites of protein synthesis. Messenger (template) RNA relays the genetic message from the DNA to the ribosomes where it becomes a part of the active protein synthesizing complex. Specific soluble RNA molecules which serve as adaptors are complexed to their specific activiated amino acids and also enter into the active ribosome complex. The base sequence of the messenger RNA serves as a template to orient the soluble RNA adaptor molecules and control the sequence of amino acids incorporated into the polypeptide chain. As protein synthesis proceeds the messenger template moves across the surface of the ribosome and the $NH_3$-terminal end of the newly formed polypeptide chain moves away from the point of peptide bond formation. The messenger RNA is unstable and functions for only a short time. Although these major mechanisms have been well established, many other aspects of protein synthesis are not known.

# MATERIALS AND METHODS

## Selection of Oat Varieties

Seven oat varieties and certain crosses between them were used in this study. Six of the varieties - Bonham, Andrew, Nemaha, Richland, Minland and Mo. 0-205 - had descending degrees of relationship to Cherokee (based on their common parentage with Cherokee). These same varieties were used by Kleese and Frey (1964). Crosses were made between Cherokee and each of the other varieties during the winter seasons between 1953 and 1955 and 90 $F_2$-derived lines were randomly selected from each cross. Thirty lines of Cherokee and five lines of each of the other varieties were also derived. The 540 $F_2$-derived and 60 parental lines were grown in a hill-plot experiment in 1958 using a randomized complete block design with four replicates. Thirty seeds were planted in each hill and hills were spaced one foot apart in perpendicular directions. The entire experiment was bordered with three rows of hills to avoid border effect. On each plot heading date was recorded when 50% of the panicles were completely emerged and plant height was recorded as the number of inches from ground level to the panicle tips. At maturity the plots were harvested and the yield of threshed grain measured. Variance components among lines for plant height, heading date and

Table 1. Expected mean squares from analysis of variances of data from each cross

| Source of Variation | Degrees of Freedom | Expected Mean Squares |
|---|---|---|
| Lines | $(n - 1)$ | $\sigma_e^2 + r\sigma_L^2$ |
| Error | $(r - 1)(n - 1)$ | $\sigma_e^2$ |

grain yield were calculated using the model in Table 1. Variance components were used as indices of genetic relationship of the oat varieties.

## Sources of Antigen

Antigens were extracted from the tissues of six plant parts--pollen, embryo, scutellum, whole grain, coleoptile and root. All seven varieties were used as sources of pollen antigens, but only four varieties--Cherokee, Nemaha, Andrew and Mo. 0-205--were used as sources of antigens of other plant parts. Nemaha, Andrew, and Mo. 0-205 were chosen to cover the full range of genetic relationship to Cherokee as determined by grain yield variance components and serological relationships determined by Kleese and Frey (1964).

Pollen was collected from drilled plots of each of the seven varieties in 1963 and 1964. In 1965 pollen was collected only from varieties for which adequate quantities were not

collected previously. The collection method described by Kleese (1962) was used. In the mornings during the anthesis season, bunches of panicles were covered by loosely tied paper bags. In late afternoon after the oat florets had opened, the bags were shaken to dislodge the pollen and carefully removed. Pollen was collected from the bags, air dried, cleaned by sifting through a 150 mesh sieve and stored in a desiccator. Grain was harvested from the same plots and stored in an air conditioned seed-storage room. It served as a source of embryo, scutellem and whole-grain antigens and was germinated to obtain root and coleoptile tissues.

The embryo and scutellum tissue were obtained by hand separation of de-hulled seeds that were soaked for one hour in distilled water. To reduce microbial contamination, the seed was treated with 10% Chlorox solution for five minutes before soaking. Tissues were air dried immediately after separation and stored at -10° F.

Coleoptile and root tissues were obtained from seedlings 12 to 14 days old. Coleoptile tissue was the vegetative portion of the seedlings and consisted of coleoptile and first leaf. Seedlings were grown in the dark to prevent chlorophyll formation. To facilitate the separation of roots from seed remnants, the seed was planted on brass screens placed in flats of moist sand and covered with a half inch of sand. Coleoptile material was cut above the sand and roots were

separated from the sand by a jet of water, then the roots were cut away from the screen. This method yielded quantities of clean roots with relatively little labor. Some root and coleoptile tissues were extracted fresh and used directly; some were frozen in liquid nitrogen and freeze-dried in a Vir-Tris tissue drier and refrigerated at 5° C.

## Antisera Production

### Guinea pig antisera

Guinea pig antisera were produced against all seven varieties of pollen antigens and against the four varieties of antigens--Cherokee, Nemaha, Andrew and Mo. 0-205--from whole grain, coleoptile, embryo, scutellum and root. The methods used in antisera production were standardized to obtain uniform antisera. Only healthy guinea pigs about five months old were used. The crude protein contents of the antigens were determined with the micro-Kjeldahl technique (total nitrogen multiplied by the factor of 6.25) and uniform amounts of protein were administered to each animal. Some plant antigens required the injection of more protein than others to stimulate antibody production. The immunization period was one month and animals that did not respond during this length of time were discarded. Longer periods of immunization were avoided because there was the possibility of losing antibody specificity (Wolf, 1955; Leone, 1952).

Antisera against pollen, embryo and scutellum were produced by immunizing guinea pigs with intramuscular injections of an antigen mixture consisting of ground plant material, buffered saline and Freund's incomplete adjuvant (Freund, 1948). A total of 15 mg of crude plant protein was injected in two equal doses two weeks apart. Each dose was injected into two sites, one in each thigh.

Other pollen antisera were produced by immunizing guinea pigs by subcutaneous injections. Each animal received a total of 30 mg of pollen protein given in two doses administered two weeks apart. Each dose of antigen mixture consisted of ground plant tissue containing 15 mg crude protein, .5 ml 0.1 M phosphate-buffered saline (pH 6.8) and .5 ml Freund's incomplete adjuvant. A dose of antigen was injected into 10 subcutaneous sites. This method of injection was not as severe on the animals as intramuscular injections and it produced antisera of good titer.

Guinea pig antisera were produced against coleoptile, whole grain and root proteins by intradermal injection. The protein extractions of root and coleoptile were made by homogenizing equal amounts of fresh plant tissues and cold 0.1 M phosphate-buffered physiological saline (pH 7.0) in a Stein mill for 30 seconds. After extracting for two hours in a refrigerator the liquid was separated from the residue by pressing through cheese cloth, and then was centrifuged at

20,000 x g at 1° C to remove remaining cell fragments.

The whole-grain extracts were prepared from ground grain that had been pre-extracted twice with ether to remove the lipids. Five ml of cold 0.1 M phosphate-buffered physiological saline (pH 6.9) was added and the mixture was refrigerated for four hours and then centrifuged as above. The extracts were concentrated to about 1/5 their original volumes by evaporation at 7° C.

Animals were immunized against coleoptile, root and grain proteins with injections of approximately 20, 15 and 36 mg of protein, respectively. The antigen mixture was prepared by mixing equal parts concentrated extract and Freund's incomplete adjuvant. Doses from 1.0 ml to 1.5 ml, depending on the protein content, were administered intradermally in ten sites.

At the time of the initial injection, five ml of blood were taken from each animal by cardiac puncture to provide a normal serum control. One month later the guinea pigs were bled by cardiac puncture. They were fasted for 20 hours prior to bleeding to reduce the serum lipid content which causes the serum to be turbid. The samples of blood were placed in clean test tubes and allowed to coagulate and then incubated for four hours at 37° C to allow the clots and serum to separate. The serum was decanted and centrifuged to remove the free cells, then frozen and stored at -20° C. The yield of serum per guinea pig varied from about 10 to 17 ml.

Rabbit antisera production

Rabbit antisera were produced against Cherokee coleoptile and whole-grain antigens by intravenous injections of crude extracts prepared in the same way as for guinea pigs. About 175 mg coleoptile protein or 230 mg whole-grain protein per animal were administered in 11 doses at the rate of three per week. The quantities of protein injected were increased from seven mg for the initial injections to 27 mg coleoptile and 35 mg whole-grain protein in the final one. Before starting the immunization, a 10 ml control(normal) blood sample was taken. The animals were bled by cardiac puncture 10 days after the last injection with approximately 50 ml blood taken on each of two days. The rabbits were fasted 12 hours previous to bleeding, the blood was processed and serum stored in the same way as the guinea pig blood.

One antiserum against Cherokee pollen was produced by intradermal injections of finely ground pollen containing 55 mg protein mixed with saline and Freund's incomplete adjuvant. Half of this protein was injected initially, the other half after two weeks.

## Antigens For *In Vitro* Tests

Crude extracts of pollen, whole grain, embryo, scutellum, coleoptile and root tissues were used as antigens for the *in vitro* tests. All extractions were made with 0.1 M phosphate-

buffered physiological saline with 0.1% sodium azide added as a preservative. The extracts were prepared when needed for *in vitro* tests and were stored in a refrigerator at 5° C until used.

Pollen extraction

Two and one-tenth grams pollen of each variety were delipified by pre-extracting twice with 30 ml cold anhydrous ether for 2 hours. The ether was decanted and the pollen air dried overnight, dry ground with a mortar and pestle and then wet ground, using part of the extraction saline, until the pollen grains were ruptured. The ground pollen was extracted with 18 ml cold buffered saline solution (pH 6.7) for four hours in the refrigerator and then centrifuged at 20,000 x g at 1° C for 30 minutes to remove the cell fragments. The protein content was determined by measuring the difference in nitrogen content of the extracts (using the micro-Kjeldahl technique) before and after the protein removal with trichloroacetic acid (a final concentration of 10% TCA). From 1/3 to 1/2 of the total nitrogen was precipitated by the trichloroacetic acid. The precipitable protein content of the extracts ranged from 0.35% to 1.0% and was standardized to 0.20%.

Coleoptile tissue extraction

Extracts of coleoptile were made from freeze-dried material ground in a Wiley mill. One and one-half grams of coleoptile from each variety were extracted with 20 ml cold buf-

fered saline solution (pH 7.0) overnight. Following this the liquid was pressed out of the residue through cheese cloth and centrifuged at 20,000 x g for 30 minutes at 1° C to remove the remaining cell fragments. The protein content of each of the extracts was determined using the Folin-phenol reagent (Lowry et al., 1951). The standard curve was prepared using known quantities of pollen protein (measured by the micro-Kjeldahl trichloroacetic acid method). The coleoptile extracts ranged in protein concentration from 0.39% to 0.54% and were standardized to 0.20%.

Root tissue extraction

Extracts were made from roots by extracting 1.5 grams of ground roots of each variety with 15 ml cold buffered saline solution (pH 7.0) overnight. The extract was separated from the residue, centrifuged and protein content determined as with coleoptile. The extracts varied in protein content from 0.20% to 0.25% and were standardized at 0.20%.

Whole grain extraction

Whole grain (including hulls) was ground in a Wiley mill and pre-extracted twice with 10 ml cold anhydrous ether per gram of ground grain for two hours each time. After air drying, 10 grams were extracted under refrigeration for four hours with 50 ml cold buffered saline solution (pH 7.0), after which the liquid was removed by centrifugation at 30,000 x g for 30 minutes at 1° C. The protein content was analyzed using

the Folin-phenol reagent. Extracts varied in protein content from 0.25% to 0.34% and all were standardized to 0.20%.

Embryo tissue extraction

The dried embryo tissue was crushed with a hammer in a metal container and delipified by pre-extracting twice for three hours each time with 25 ml cold anhydrous ether per gram of embryo tissue. The embryo tissue was air dried, re-ground with a mortar and pestle, and extracted under refrigeration with 10 ml cold buffered saline solution (pH 6.8) per gram of tissue for three hours. The extract and residue were separated by centrifuging at 25,000 x g for 30 minutes at 1° C. Because of the small amount of material available (0.7 g to 1.8 g) the residue was re-extracted with five ml of the same saline per gram of embryo. In spite of the pre-extraction to remove lipids, the extracts were quite turbid. Protein determinations were made on both extractions using the Folin-phenol reagent. The protein concentrations ranged from 0.8% to 1.1% in the first extraction, and from 0.3% to 0.45% in the second extraction. The extracts were pooled and diluted to a standard protein concentration of 0.40%. To remove the turbidity present in some extracts, they were recentrifuged at 25,000 x g for 30 minutes. The turbidity concentrated near the top of the centrifuge tube and the clear portion under this layer was removed with a pipette. This treatment cleared the extracts sufficiently to make them satisfactory for use.

Scutellum tissue extraction

Scutellum tissue was extracted using the same procedures as for embryo except the two pre-extractions were each six hours long. Seven ml buffered saline was used for each gram of scutellum. A second extraction was not necessary. The protein content of the extracts ranged from 0.85% to 1.02% and was standardized at 0.38%. Turbidity in certain extracts was removed in the same way as with embryo.

## Comparative Serological Tests

Quantitative tube precipitin tests

The method outlined by Boyden and DeFalco (1943) with the modifications applied by Kleese (1962) was used for the quantitative serological comparisons of the varieties. This method utilizes a photoelectric instrument to measure the magnitude of heterologous and homologous precipitin reactions. To get good comparative measurements of the magnitude of the precipitin reaction, series of doubling dilutions of antigen covering the entire reaction range from antigen excess to antibody excess (where possible) were titrated with constant antiserum.

One-half ml of the proper antiserum dilution was mixed with 1.0 ml of each antigen dilution in 10 x 75 mm culture tubes. Immediately after adding the antiserum the tube was inverted to mix the contents and the initial turbidity was

measured. Tubes were not inverted too rapidly because the formation and inclusion of air bubbles would give erroneous readings. Tubes were wiped before each reading. Following the initial reading, the tubes were incubated in a water bath at 30° C for one hour and another turbidity reading was taken. The difference between the two readings was the magnitude of the reaction.

Turbidity readings were made in a Coleman Model 9 Nephlo-Colorimeter with an immersion well modified to accommodate the culture tubes. It was also adapted with flow-through water connections to allow continuous replacement of distilled water in the immersion well. This maintained a constant temperature and prevented accumulation of lint from the tubes.

Only scratch-free, clean Pyrex culture tubes that gave consistent turbidity readings with a uniform suspension of bentonite were used. Care was taken to always place the tubes in the immersion well the same way.

The precipitating capacity of the antisera was predetermined by titrating 0.5 ml serial dilutions of antisera with 1 ml homologous antigen. An antigen concentration of 250 ug protein per ml was used to titrate the pollen, coleoptile, embryo and scutellum antisera, and a concentration of 375 ug protein per ml was used for whole-grain antisera. Using this data, the antisera were diluted as nearly as possible to a uniform precipitating capacity.

All antisera were diluted with 0.1 M phosphate-buffered physiological saline (pH 6.8) and all antigen extracts were diluted with 0.05 M phosphate-buffered saline (pH 6.8) containing 0.5% sodium chloride.

The serological relationships among the oat varieties were studied with each plant part except root. Sufficient quantities of good quality root antisera were not available to make complete comparisons. Four Cherokee antisera (i.e., antisera from four guinea pigs) were used to titrate antigens of each variety (Method A). Reciprocal comparisons were made using two antisera of each variety to titrate the homologous and Cherokee antigens (Method B). Controls of each dilution of antigen and of each antiserum also were assayed for turbidity change during incubation. The reaction readings were adjusted for turbidity in the controls. Normal serum controls were also assayed to detect non-specific reactions.

Calibration and adjustment of the nephlometer

The Coleman Model 9 Nephlo-Colorimeter was calibrated using a barium sulfate suspension equivalent to the number one tube of the McFarland Nephlometer (McFarland, 1907). (The reference suspension consisted of 1 ml of 1% barium chloride solution and 99 ml of 1% sulfuric acid.) The instrument was adjusted so that the turbidity of this reference was 100 galvanometer units.

To reduce instability of the nephlo-colorimeter caused by

power fluctuations, two 6-volt batteries were used as a power source. The power output from these batteries tended to dissipate rapidly at first then gradually with use. To reduce this effect, the instrument was turned on 30 minutes before readings were made. Even so, it was often necessary to adjust the instrument to the reference standard.

To give more comparable data and reduce the frequency of nephlometer adjustments the tubes were arranged in a randomized complete block experimental design. The tubes of one dilution in a comparison made up a block and the tubes were randomized within the block. For the second dilution a second block was used, etc. The nephlometer was adjusted to the reference standard before each comparison. The turbid suspensions used as reference standards were unstable, i.e., particles gradually aggregated to form larger particles that gave irregular readings. Bentonite reference standards were more stable than barium sulfate, but still had to be replaced often.

## Double Diffusion Gel Technique

The gel diffusion technique described by Ouchterlony (1948, 1949a and 1949b) with slight modifications was employed to study qualitative antigen differences among the oat varieties within each plant part and among the different plant parts of a variety. Agar was prepared by dissolving Oxoid Ionagar No. 2 (Consolidated Laboratories, Inc. Chicago Heights,

Illinois), to a concentration of 0.8% in 0.1 M phosphate-buffered physiological saline with 1.0% sodium azide added as a preservative. Five ml of agar (a 2.5 mm layer) were poured into disposable plastic petri dishes five cm in diameter. After the agar had set, a center well was cut into each plate with a No. 3 cork borer and six wells were cut around the center well with a No. 2 cork borer. The outer wells were six mm from the center well and seven mm from each other.

Undiluted antisera was placed in the center well and antigens of the appropriate varieties or plant parts (standardized to 0.2% protein content) were placed in the six surrounding wells. The plates were incubated at room temperature in a moisture-saturated atmosphere for 48 to 72 hours.

Comparisons were made of pollen antigens from the seven oat varieties using three Cherokee-pollen antisera. Triplicate comparisons were also made of four varieties--Cherokee, Nemaha, Andrew and Mo. 0-205 against Cherokee antisera for each of the other plant parts. Cherokee pollen, whole grain, embryo, scutellum, root and coleoptile antigens were compared in duplicate against Cherokee antisera for each plant part. Representative plates of the comparisons were photographed.

# RESULTS

## Genetic Relationship

The variance components among $F_2$-derived lines within the oat crosses--for grain yield, heading date and plant height--and the coefficient of relation of the parents of the six oat crosses (calculated from putative pedigrees) are presented in Table 2. These variance components are indices of genetic relationship of each of the six varieties--Nemaha, Bonham, Richland, Andrew and Mo. 0-205--to Cherokee since crosses between parents that are genotypically diverse generally show more variability in their $F_2$ progenies than do crosses between slightly diverse parents. The variance components of each character place the varieties in a different order of genetic relationship to Cherokee. This is expected, however, since each character is based on a different set of genes. Grain yield depends upon all genetically controlled biochemical processes throughout the life of the plant and therefore, probably is based on nearly all genes of a genotype. For this reason, the within cross variance components for grain yield are considered the best estimators of genetic relationship between parent varieties.

The coefficients of relation based on the pedigrees of the parents were inversely related to the variance components. The parental relationships based on grain yield variance components

Table 2. Within cross variance components among $F_2$-derived lines for three quantitative characters and the coefficients of relation of parents of six oat crosses

| Cross | Degrees of Freedom | Characters | | | Coefficient of Relation of Parents |
|---|---|---|---|---|---|
| | | Plant Height | Heading Date | Grain Yield | |
| Cherokee x Nemaha | 89 | 0.12 | 0.00[a] | 0.25 | 0.25 |
| Cherokee x Bonham | 89 | 0.08 | 0.06 | 3.12** | 0.50 |
| Cherokee x Richland | 89 | 2.32** | 5.65** | 5.05** | 0.25 |
| Cherokee x Andrew | 89 | 0.52** | 0.77** | 14.22** | 0.375 |
| Cherokee x Minland | 89 | 1.64** | 1.09** | 18.92** | 0.0625 |
| Cherokee x Mo. 0-205 | 89 | 4.74** | 4.12** | 18.75** | 0.0625 |
| Error | 267 | | | | |

**Exceeds the 1% level.

[a]Negative variance assumed to be 0.00

and the coefficients of relation agreed with two exceptions. First, the coefficients of relation of Nemaha to Cherokee, 0.25, was too low. It is generally accepted among oat breeders of the Midwest that these two varieties are very closely related and might even be the same genotype. A mistake may have been made in record keeping during their development. All evidences--agronomic characteristics, variances within crosses between Cherokee and Nemaha, and serological comparisons--

support this theory. Second, the relationship of Richland and Andrew to Cherokee according to grain yield variance components was the reverse of the coefficient of relation. Of course, the coefficient of relation was based on the assumption of random distribution of genes from common parents, but the actual sample of genes that a given variety contains may give actual genetic relationships different from those predicted. This may account for the discrepancies of Andrew and Richland.

## Quantitative Serological Comparisons

The values from the quantitative precipitin reactions were all expressed relative to the values from the homologous reaction. The homologous reaction was used as the standard for each antiserum and was the base to which all heterologous reactions with the same antiserum were referred. The sum of the heterologous reactions were divided by the sum of the homologous reactions to give the serological relationships (Heterologous turbidity ÷ Homologous turbidity x 100 = % serological relationship).

### Pollen comparisons

The use of crude pollen extracts as antigens for the *in vitro* tests presented no special problems. Essentially the complete reaction range of these antigens was titrated and non-specific reactions (reactions between antigen and normal serum)

were not observed. Typical titration curves of pollen antigens are shown in Figure 1. Anti-Cherokee serum was used to titrate Cherokee and Andrew pollen antigens. The sum of the Cherokee and Andrew reactions (sums of turbidities) were 76 and 45, respectively; thus the serological relationship of Andrew to Cherokee is 59% (Heterologous turbidity ÷ Homologous turbidity x 100).

Table 5 (Appendix) shows the sums of turbidities for four Cherokee antisera used to titrate the pollen antigens of the seven oat varieties (Method A) and reciprocal comparisons using two antisera of each variety to titrate Cherokee and the homologous antigens (Method B). The serological relationships of the six varieties to Cherokee as estimated by Method A (the Cherokee antisera) and Method B (the reciprocal comparisons) are given in Table 6 (Appendix).

Antisera produced by subcutaneous and intramuscular injection gave somewhat different results, i.e., subcutaneous injection generally produced lower serological relationships. Cherokee antisera from guinea pigs 1 and 2 (Method A) and the number 1 antisera (guinea pig) of Andrew, Nemaha and Mo. 0-205 (Method B) were produced by subcutaneous injections (Tables 5 and 6). The other pollen antisera were produced by intramuscular injections. The means of the serological relationships predicted from Method A and B and the within cross variance components are summarized in Figure 2. The two serologi-

Figure 1. Typical titration curves of Cherokee and Andrew pollen extracts titrated with Cherokee pollen antiserum. The antigen concentration of dilution No. 1 was 1:500 (Protein:diluent)

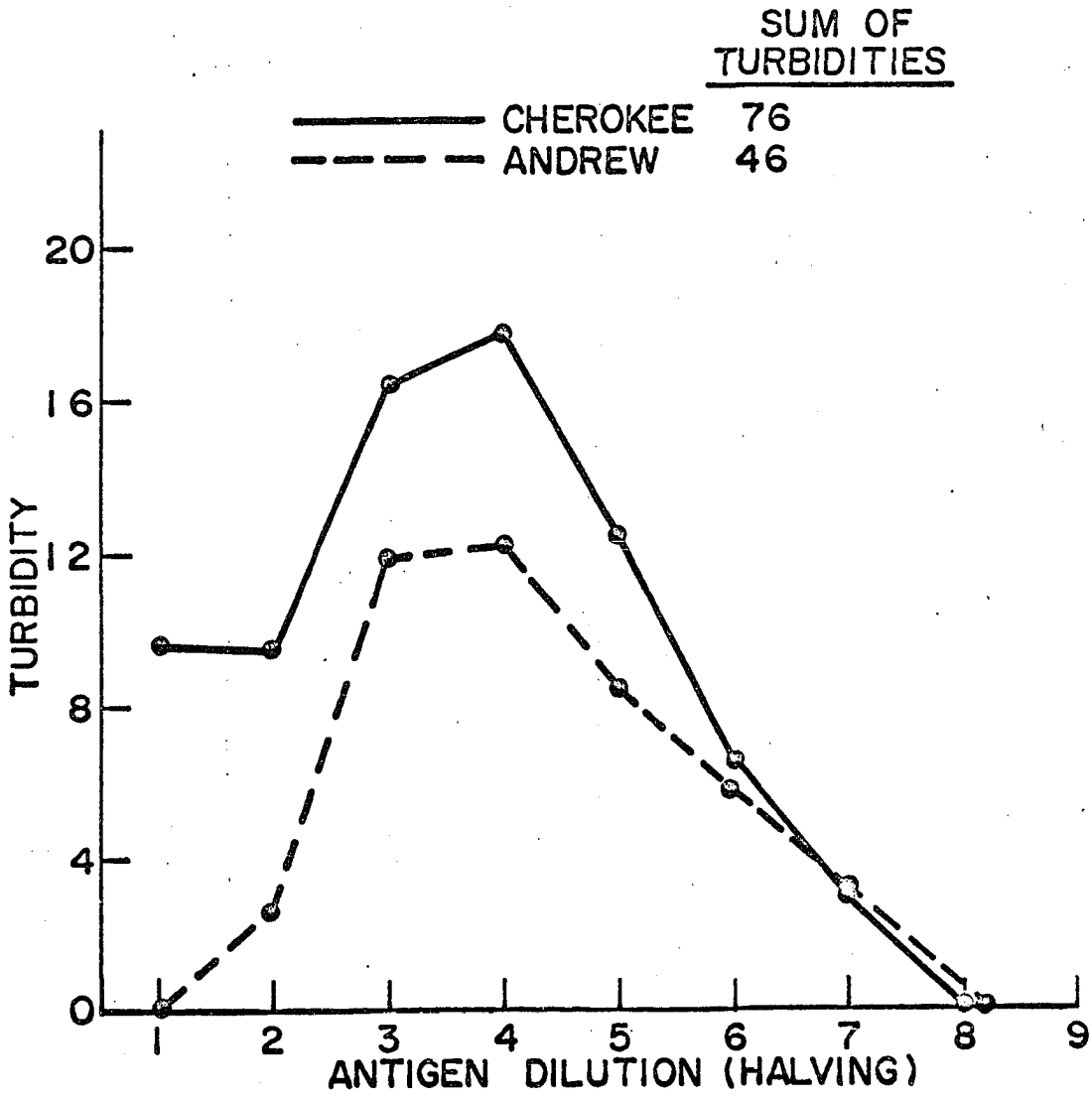
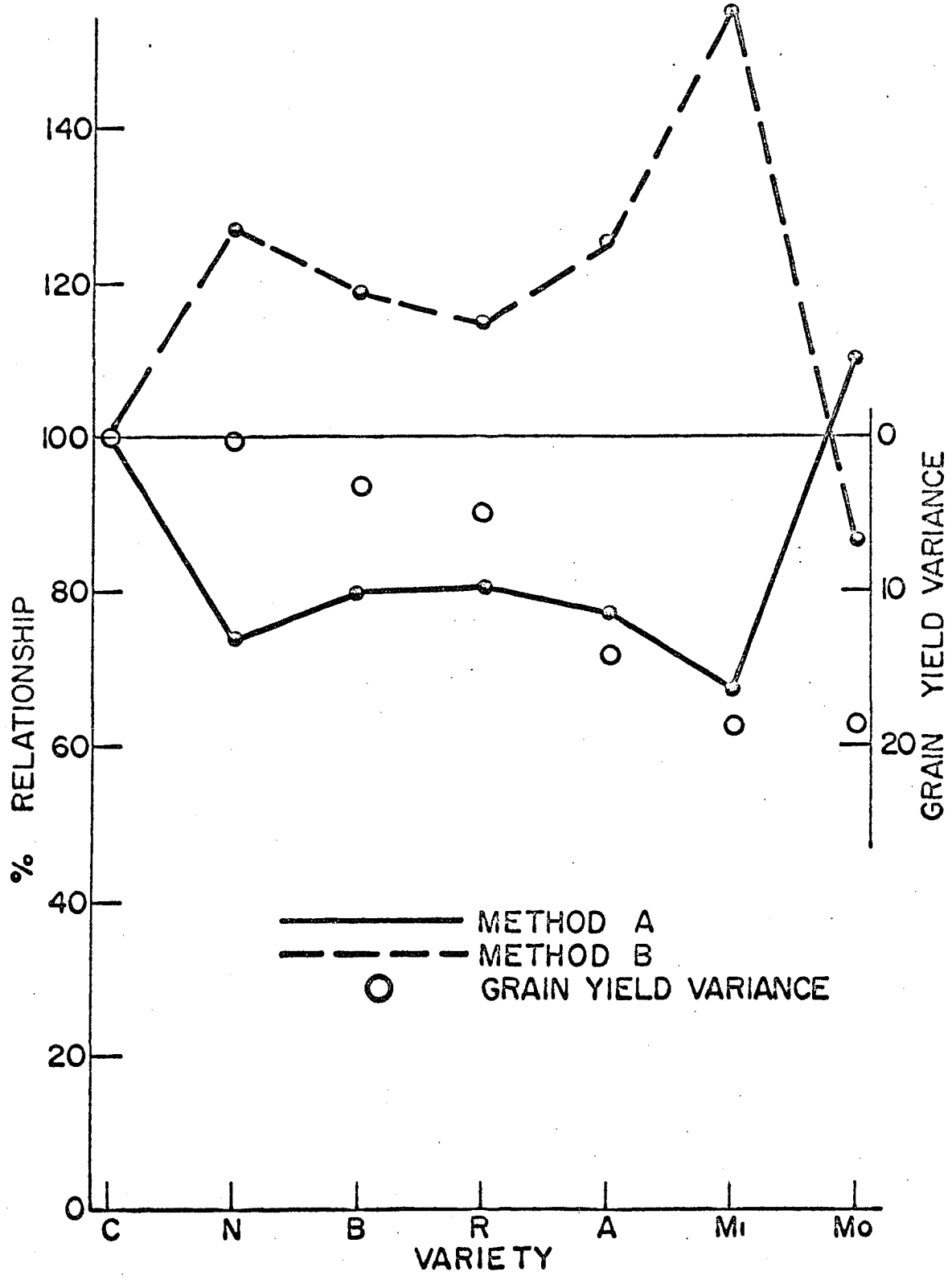

SUM OF
TURBIDITIES
CHEROKEE 76
ANDREW 46
TURBIDITY
0
4
8
12
16
20
1
2
3
4
5
6
7
8
9
ANTIGEN DILUTION (HALVING)

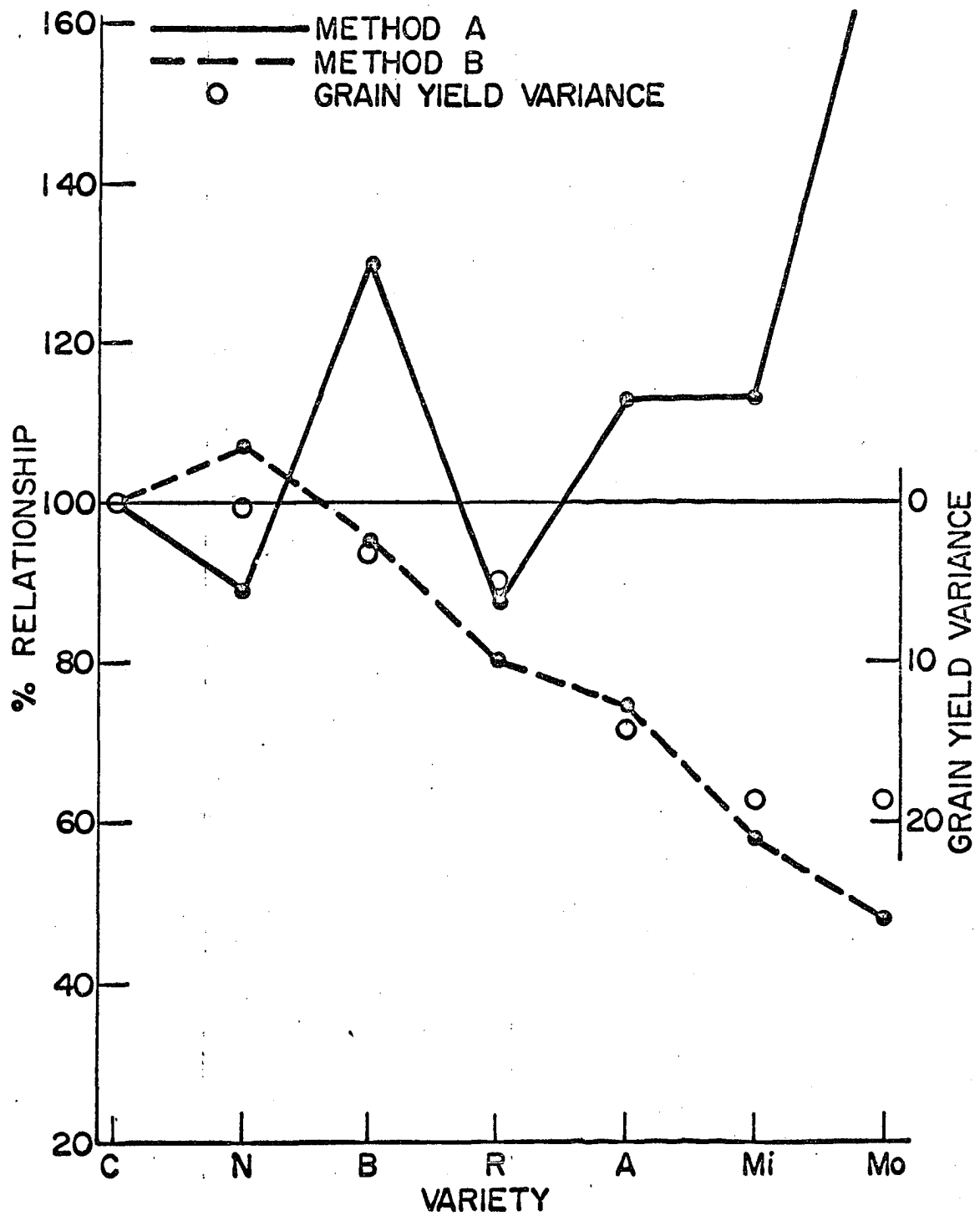
Figure 2. The mean pollen serological relationships of six oat varieties to Cherokee estimated by Method A and Method B and to grain yield variance components (plotted in an inverse order, right ordinate). Varieties on abscissa are in order of decreasing genetic relationship to Cherokee based on the grain yield variance components. C = Cherokee, N = Nemaha, B = Bonham, R = Richland, A = Andrew, Mi = Minland and Mo = Mo. 0-205


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
METHOD A
METHOD B
GRAIN YIELD VARIANCE
C
N
B
R
A
Mi
Mo
VARIETY

cal methods, A and B, produced reciprocal results, i.e., the lines which connect the relationship percentages are virtually mirror images of one another with center at 100. This was unexpected because theoretically with either method all relationships should be less than 100%. Actually Method A produced estimates of relationship that agreed, to a certain extent, with the relationships estimated by the grain yield variance components. The exceptions being Cherokee and Nemaha, and Cherokee and Mo. 0-205. However, there is no a priori reason to accept these serological relationships in preference to those from Method B.

Since one purpose of this study was to attempt to confirm the conclusions of Kleese and Frey (1964), their data are summarized graphically in Figure 3. They concluded that the genetic relationships of the six oat varieties to Cherokee could be predicted as satisfactorily with serological data as with grain yield variance components. Although their data do not agree with mine in all respects, two similarities exist, (a) relationships estimated by Methods A and B are reciprocal and, (b) many relationships estimated by one method are above 100%. The two studies differ in that reasonable relationships in their study were produced by Method B, in mine by Method A. Their Method B results agreed very well with the grain yield variance components.

Figure 3. The mean pollen serological relationships (from Kleese 1962) of six oat varieties to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties on abscissa are in order of descending genetic relationship to Cherokee as estimated by the grain yield variance components. C = Cherokee, N = Nemaha, B = Bonham, R = Richland, A = Andrew, Mi = Minland and Mo = Mo. 0-205


METHOD A
METHOD B
GRAIN YIELD VARIANCE
160
140
120
100
80
60
40
20
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
C
N
B
R
A
Mi
Mo
VARIETY

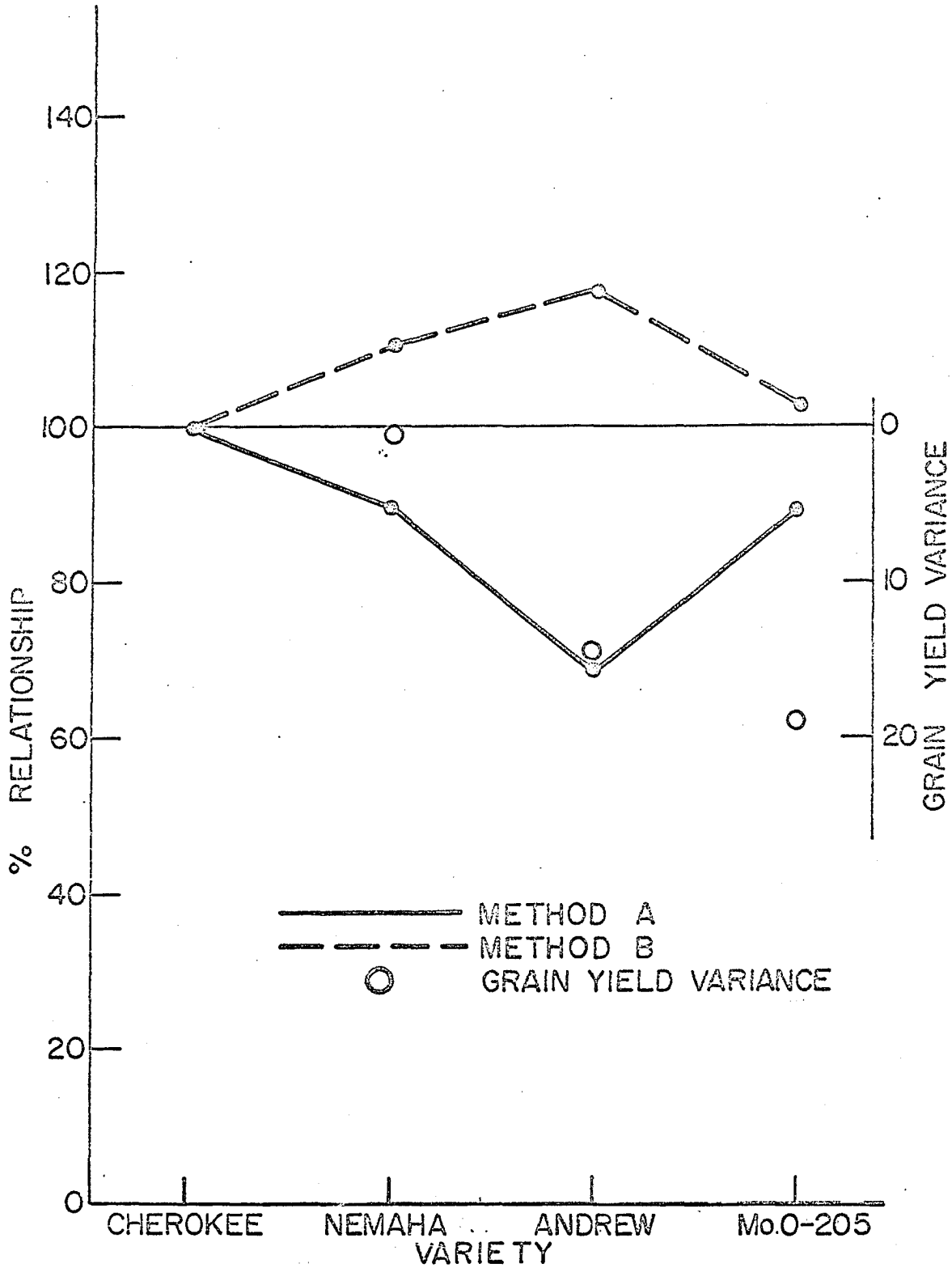
Coleoptile comparisons

The use of crude coleoptile extracts as antigens for *in vitro* tests caused two special problems: (a) non-specific reactions were observed, and (b) the complete reaction range was not titratable. To titrate the complete reaction range the antigen dilution series must begin with a concentration high enough to cause complete antigen inhibition of the reaction. With coleoptile extracts, when the antigen concentration was increased to the inhibition zone, non-specific reactions offset the decrease in reaction due to antigen inhibition. Since non-specific reactions were undesirable, the highest antigen concentration producing no significant non-specific reaction was used as the first tube in the dilution series. As a result, the complete reaction range was not titrated (Figure 16 in Appendix).

The sums of turbidities of each titration of the four varieties--Cherokee, Nemaha, Andrew and Mo. 0-205--are presented in Table 7 (Appendix). Table 8 (Appendix) presents the serological relationships of the three varieties to Cherokee. Figure 4 shows the serological relationships estimated by Methods A and B and the within cross grain yield variance components.

As with the pollen, the relationships estimated by Methods A and B were reciprocal to one another. With Method B the relationships of Nemaha and Andrew to Cherokee were considerably

Figure 4. The mean coleoptile serological relationships of Nemaha, Andrew and Mo. 0-205 to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties on abscissa are in order of descending genetic relationship to Cherokee as estimated by the grain yield variance components


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
METHOD A
METHOD B
GRAIN YIELD VARIANCE
CHEROKEE
NEMAHA
ANDREW
Mo.0-205
VARIETY

above 100%, whereas similar relationships estimated by Method A agreed well with those estimated by the variance components. The relationship of Mo. 0-205 to Cherokee was higher than expected.

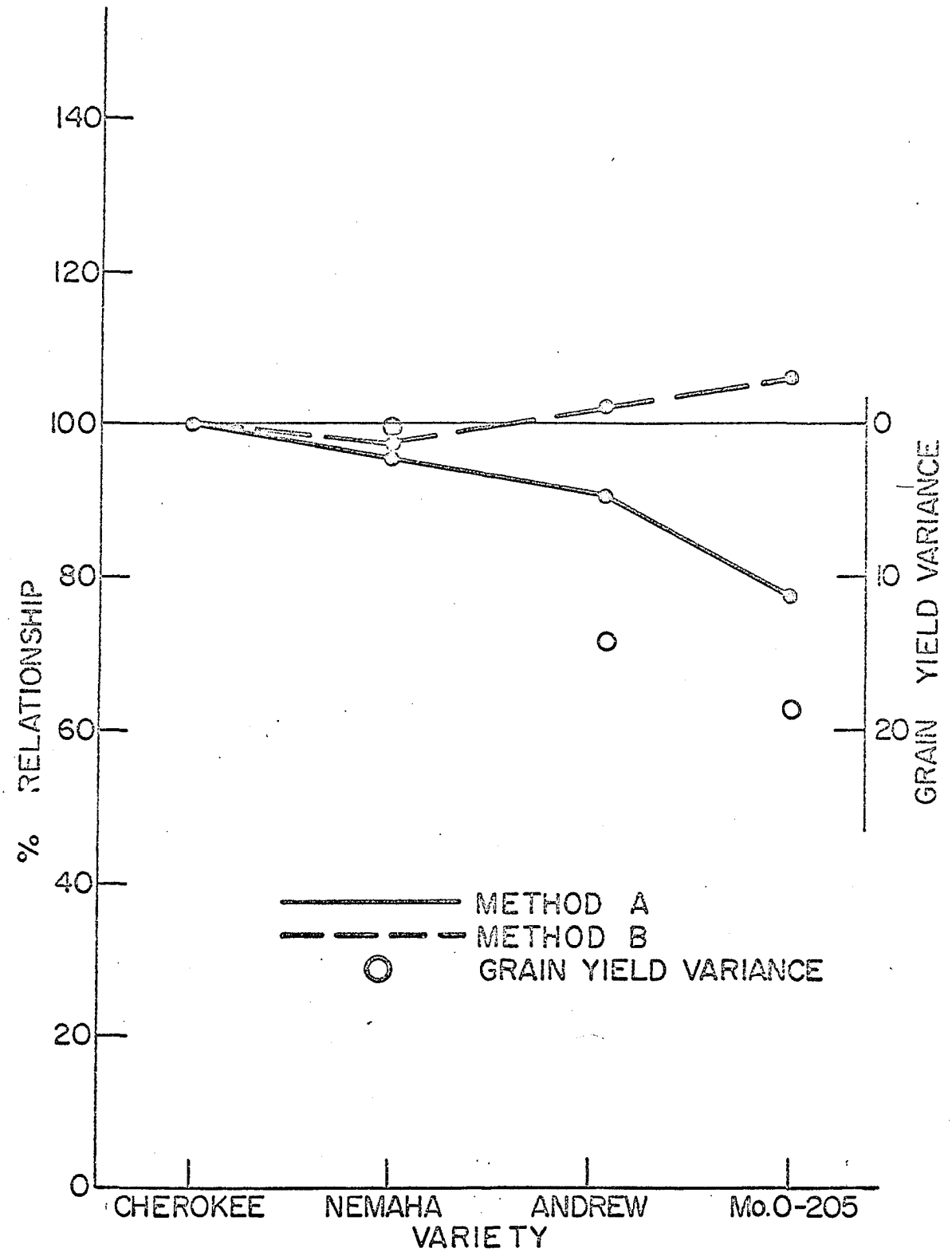
Both Methods A and B were expected to give similar results, but obviously did not.

Whole grain comparisons

Crude whole-grain extracts, like crude coleoptile extracts, gave considerable non-specific reactions when the initial antigen concentration of the serial dilution series was increased to the antigen inhibition zone. These reactions were avoided, as with coleoptile, by not titrating the entire reaction range of the antigens (Figure 17 in Appendix). The sums of turbidities and serological relationships for whole-grain antigen-antibody reactions are given in Tables 9 and 10 (Appendix). A summary of the serological relationships and the within cross variance components for grain yield are given in Figure 5.

As with pollen and coleoptile antigens, Methods A and B produced different estimates of relationships among the oat varieties when whole-grain antigens were used. The relationships estimated by Method A and the variance components agreed. Although the relationships estimated by Method B did not agree with those of Method A, none was much above 100%.

Figure 5. The mean whole-grain serological relationships of Nemaha, Andrew and Mo. 0-205 to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties on abscissa are in order of descending genetic relationship to Cherokee as estimated by the grain yield variance components


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
METHOD A
METHOD B
GRAIN YIELD VARIANCE
CHEROKEE
NEMAHA
ANDREW
Mo.0-205
VARIETY

Scutellum comparisons

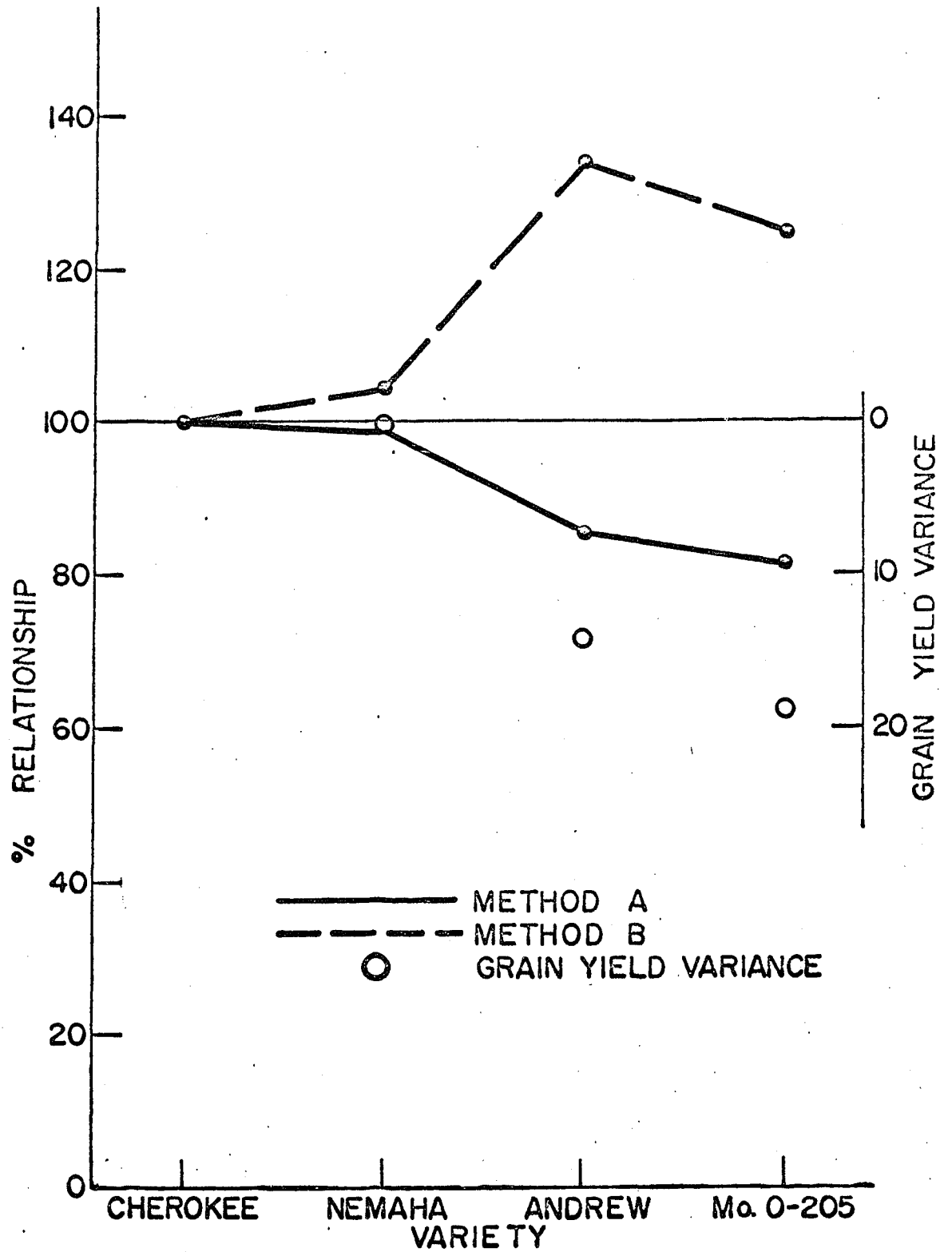
There were some special problems when crude scutellum extracts were used as antigens for *in vitro* tests. First, intensive non-specific reactions occurred. These were corrected by titrating each antigen with the normal control of each antiserum and subtracting the resulting turbidity from the antigen-antisera reaction turbidity reading. Second, the complete reaction range of the antigen could not be titrated (Figure 18 in Appendix). Table 11 (Appendix) shows the total adjusted turbidities for the scutellum antigen reactions (normal reactions subtracted), and Table 12 (Appendix) shows the serological relationships of the three varieties with Cherokee. The mean serological relationships and the within cross variance components for grain yield are summarized in Figure 6.

Method B estimated unreasonable mean relationships of Andrew and Mo. 0-205 to Cherokee of 134 and 125%. However, the estimates of relationship among the oat varieties from the grain yield variance components and Method A serological readings were quite similar.

Embryo comparisons

The non-specific reactions of crude embryo extracts were even more intense than with scutellum extracts. To correct for the non-specific reactions, the crude embryo extracts were titrated with samples of normal control sera and the turbidities subtracted from the total turbidities of antigen

Figure 6. The mean scutellum serological relationships of Nemaha, Andrew and Mo. 0-205 to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties on abscissa are in order of descending genetic relationship to Cherokee as estimated by the grain yield variance components


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
METHOD A
METHOD B
GRAIN YIELD VARIANCE
CHEROKEE
NEMAHA
ANDREW
Mo. 0-205
VARIETY

and antisera reactions. The complete reaction range of the antigens could not be titrated here either (Figure 19 in Appendix). Tables 13 and 14 (Appendix) present the total turbidities and serological relationships of the embryo comparisons. A summary of the mean serological relationships of the three oat varieties with Cherokee and the within cross variance components for grain yield is given in Figure 7. The relationships estimated by Method A varied around 100% in a random fashion, and those estimated by Method B ranged between 160 and 200%. Neither method produced reasonable nor useful results.

Another type of embryo experiment was conducted by titrating dilutions of antisera with constant antigen at a concentration low enough to avoid non-specific reactions. Typical curves of these titrations are shown in Figure 20 (Appendix) and the sums of turbidities and the serological relationships are shown in Tables 15 and 16 (Appendix). A summary of the relationships among oat varieties estimated from the dilutions of antisera and grain yield variance components is given in Figure 8. These relationships followed the same trends as those shown from other plant parts, i.e., Method A did not agree with Method B, and there was a reciprocal relationship between the two methods. The serological relationships estimated by Method A agreed very well with those estimated by the grain yield variance components, but

Figure 7. The mean embryo serological relationships of Nemaha, Andrew and Mo. 0-205 to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties on abscissa are in order of descending genetic relationship to Cherokee as estimated by the grain yield variance components

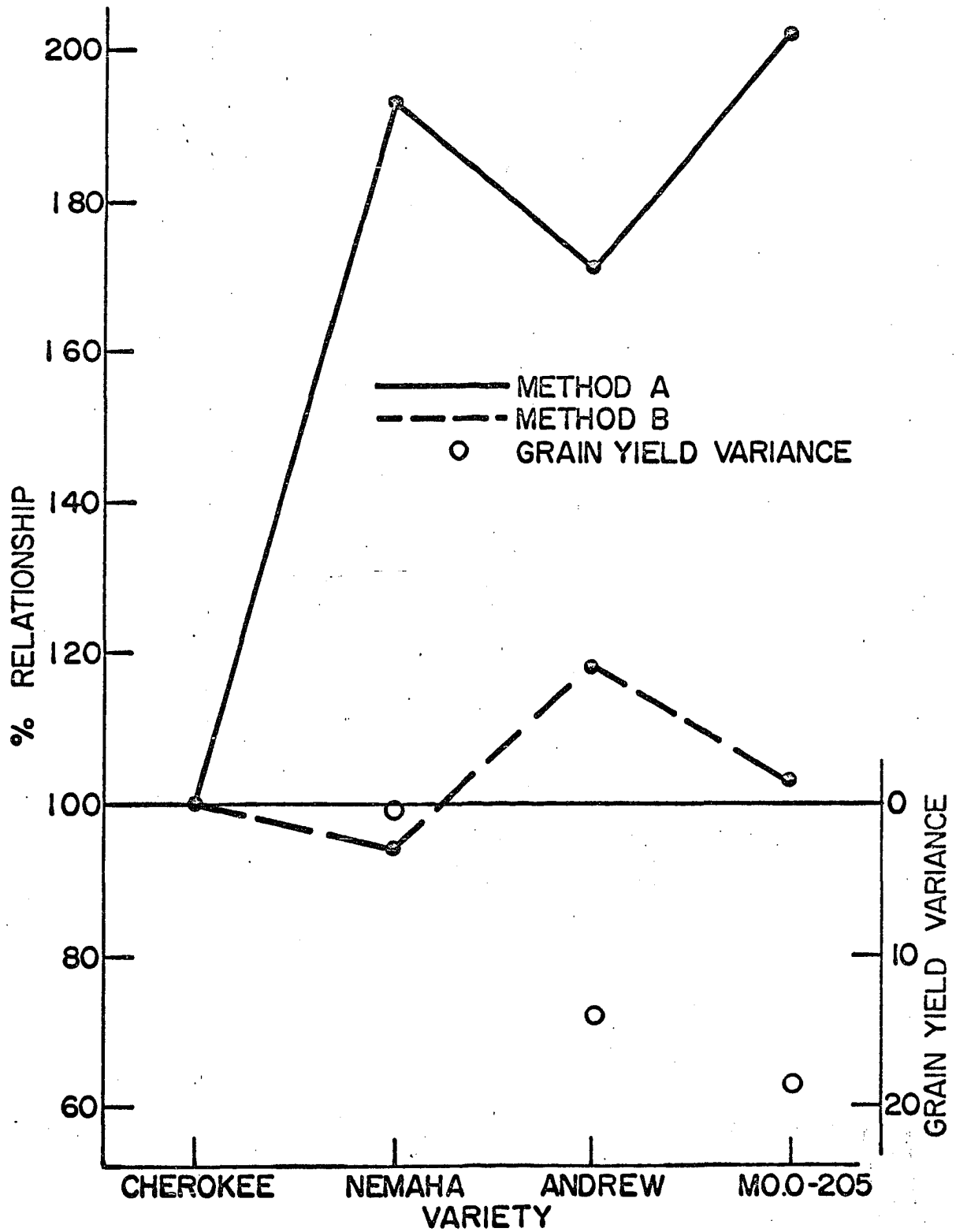
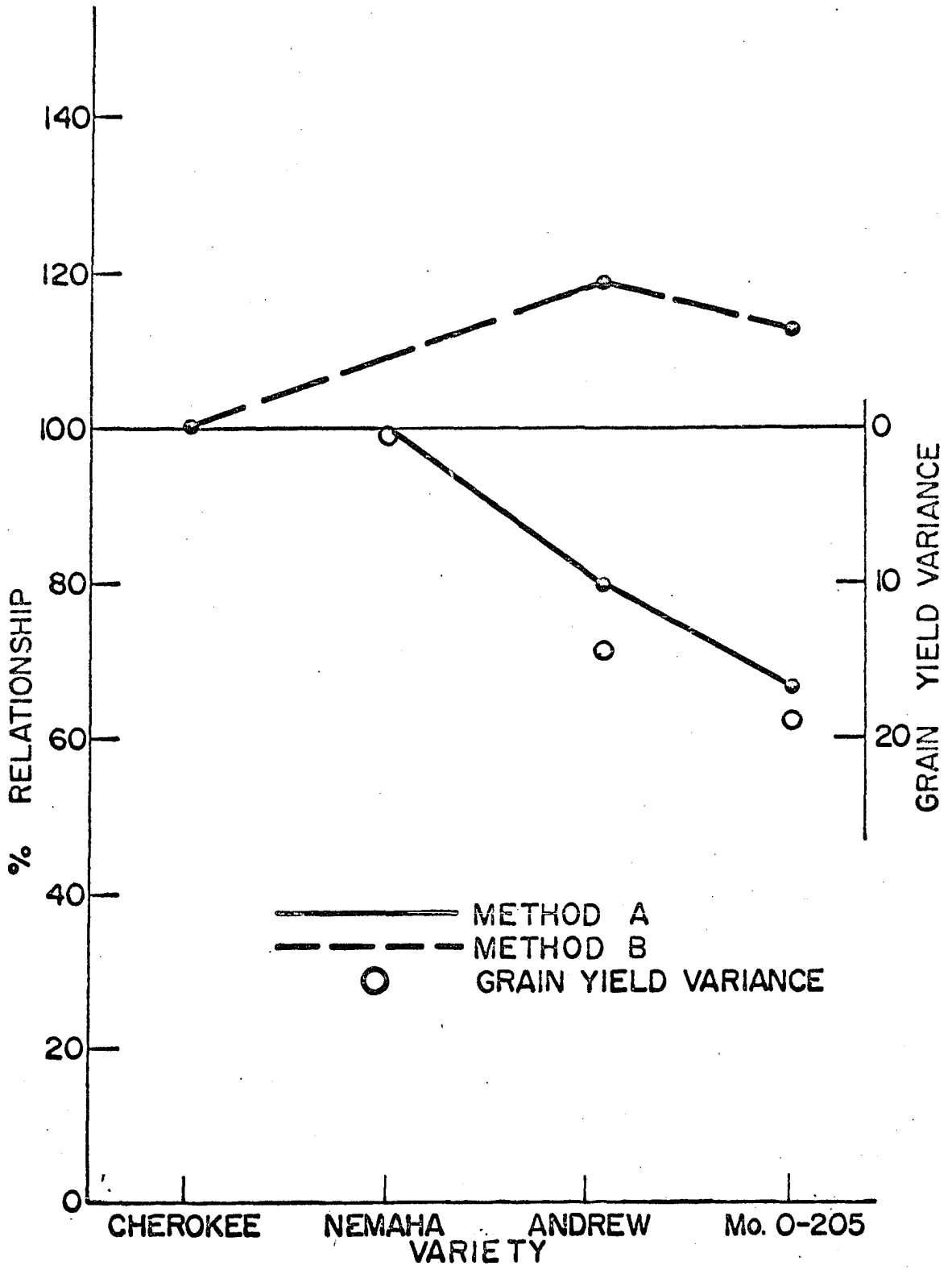

200
180
160
140
120
100
80
60
% RELATIONSHIP
METHOD A
METHOD B
GRAIN YIELD VARIANCE
0
10
20
GRAIN YIELD VARIANCE
CHEROKEE
NEMAHA
ANDREW
MO.0-205
VARIETY

Figure 8. The mean embryo serological relationships (from comparisons using serial dilutions of antisera titrated by constant antigen) of Nemaha, Andrew and Mo. 0-205 to Cherokee estimated by Method A and Method B and the grain yield variance components (plotted in inverse order, right ordinate). Varieties plotted on the abscissa in a descending order of genetic relationship to Cherokee as estimated by the grain yield variance components


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
METHOD A
METHOD B
GRAIN YIELD VARIANCE
CHEROKEE
NEMAHA
ANDREW
Mo. 0-205
VARIETY

the relationships from Method B were all above 100%.

Even though the results of the quantitative precipitin tests using antisera from guinea pigs did not estimate genetic relationships among oat varieties as expected, the patterns established by antigens from all plant parts were similar. The two serological methods, A (antigens from all varieties titrated with Cherokee antiserum) and B (antisera from the various oat varieties titrated with antigens from Cherokee and the respective varieties) usually estimated reciprocal serological relationships. The relationships of other oat varieties to Cherokee estimated serologically by Method A generally were similar in trend to those estimated from the grain yield components of variance. Since the relative magnitudes of the within-cross variance components of grain yield for a series of crosses is a standard method for estimating the genetic relationships among the parent varieties, it would seem that Method A is a good choice for estimating varietal relationships serologically. However, this is obviously a hazy approach because Kleese and Frey (1964) found that Method B estimated relationships among oat varieties correctly whereas Method A did not. Serologically both methods are equally valid for measuring genetic relationship and, in fact, should produce the same relationships. There is no justification for selecting one method in preference to the other. The consistant patterns of the two methods with all

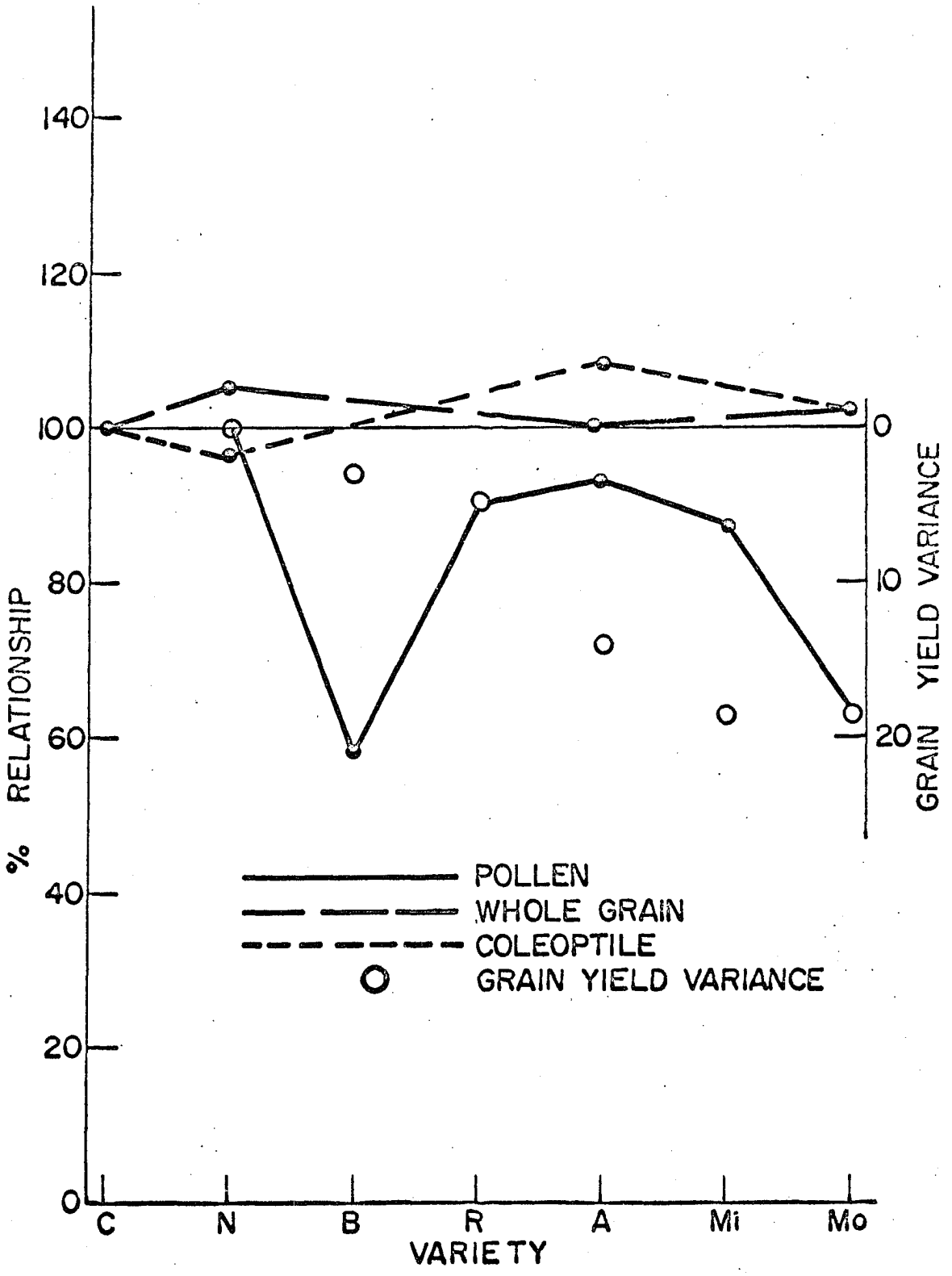
plant parts indicates that the contradictory results of Methods A and B are real and must be explained.

Although whole-grain and embryo (antisera dilutions) relationships predicted by Method A agreed well with genetic relationships estimated by the grain yield variance components, the relative value of these plant parts for predicting genetic relationships is also confused by the contradictory results of Methods A and B. Until this problem can be resolved the relative values of the plant parts can not be accurately determined.

Rabbit antisera comparisons

Four variety comparisons were made using rabbit antisera, one using pollen antigens from the seven varieties against Cherokee pollen antiserum, two using whole-grain antigens from Cherokee, Nemaha, Andrew and Mo. 0-205 varieties against Cherokee whole-grain antisera, and one using coleoptile antigens of Cherokee, Nemaha, Andrew and Mo. 0-205 varieties against Cherokee coleoptile antisera. The sums of turbidities and the serological relationships from these comparisons are given in Tables 17 and 18 (Appendix). Figure 9 gives a graphic summary of these relationships and the grain yield variance components. All of the relationships estimated from whole-grain and coleoptile antigens were very close to 100%. The relationships estimated by pollen antigens were all below 100% and to a certain extent agreed with the grain yield variance components.

Figure 9. The mean serological relationships obtained from pollen, whole-grain and coleoptile antigens reacting with rabbit Cherokee antisera (Method A only) and the grain yield variance components (plotted in inverted order, right ordinate). Varieties on the abscissa are in descending order of genetic relationship to Cherokee as estimated by the grain yield variance components. C = Cherokee, N = Nemaha, B = Bonham, R = Richland, A = Andrew, Mi = Minland and Mo = Mo. 0-205


140
120
100
80
60
40
20
0
% RELATIONSHIP
0
10
20
GRAIN YIELD VARIANCE
POLLEN
WHOLE GRAIN
COLEOPTILE
GRAIN YIELD VARIANCE
C
N
B
R
A
Mi
Mo
VARIETY

Since only Method A was used, it is not possible to judge whether the data from rabbit and guinea pig antisera would be similar throughout. Comparison of the trends in Figure 9 with those of Figures 2, 4 and 5 will confirm that the relationships estimated by the rabbit antisera (Method A) are not the same as those estimated by guinea pig (Method A).

## Gel Diffusion

The serological reactions of gel diffusion tests are manifested by bands of precipitate deposited where an antigen and its homologous antibody meet in the proper proportions to react. The various antigens and antibodies of a system have different diffusion rates, and consequently, over time they become separated in different zones of the gel. Therefore each antigen-antibody component of a system may form a separate band in the gel. The number of bands produced by an antigen-antibody system indicates the minimum number of antigen-antibody components present.

According to Ouchterlony (1958), the way the precipitate bands of one antigen-antibody system interact with the bands of another system tells whether or not the antigens involved are identical in the two systems. Antigens identical in two systems produce an interference at the junction of the precipitate bands, i.e., a rounding of the corners occurs where the two meet. If the antigens are different interference is

absent and the precipitate bands cross each other producing spurs of precipitate behind the junction point.

Comparison of variety antigens

Figures 10 and 11 show representative gel diffusion plates with reactions of antigens from the various oat varieties against Cherokee antisera for all plant parts. When pollen antigens from six varieties were tested against Cherokee pollen antisera, a broad band of precipitate made up of several narrower bands was formed (Plates 1 and 2 of Figure 10). The bands which represent specific-antigen antibody reactions did not separate well. This poor separation made interpretation difficult, however, the actual plates showed better resolution of the bands than did the photographs. Of the three comparisons of this type that were made, two showed three bands and the other showed two. Apparently, all detectable antigens were common to all varieties because there was interference where the bands of one variety joined another and no evidence of spurs. These data do not mean that qualitative differences in antigens of the different varieties are absent, but simply that at least three antigens common to all varieties contribute the major portion of the total serological reaction.

When coleoptile antigens of the four varieties--Cherokee, Andrew, Mo. 0-205 and Nemaha--were tested against Cherokee coleoptile antiserum, at least five bands were visible indicating at least five active antigens (Plate 3 of Figure 10).

Figure 10. Photographs of representative gel diffusion plates showing reactions of antigens of pollen coleoptile and whole-grain from various varieties with Cherokee antiserum. Antiserum placed in center well and variety extracts in outer wells. (Letters near wells designate extract, A = Andrew, B= Bonham, C = Cherokee, C' = another Cherokee extract, L = Minland, M = Mo. 0-205, N = Nemaha and R = Richland

Plate 1 and 2. Pollen extracts and antiserum

Plate 3. Coleoptile extracts and antiserum

Plate 4. Whole-grain extracts and antiserum

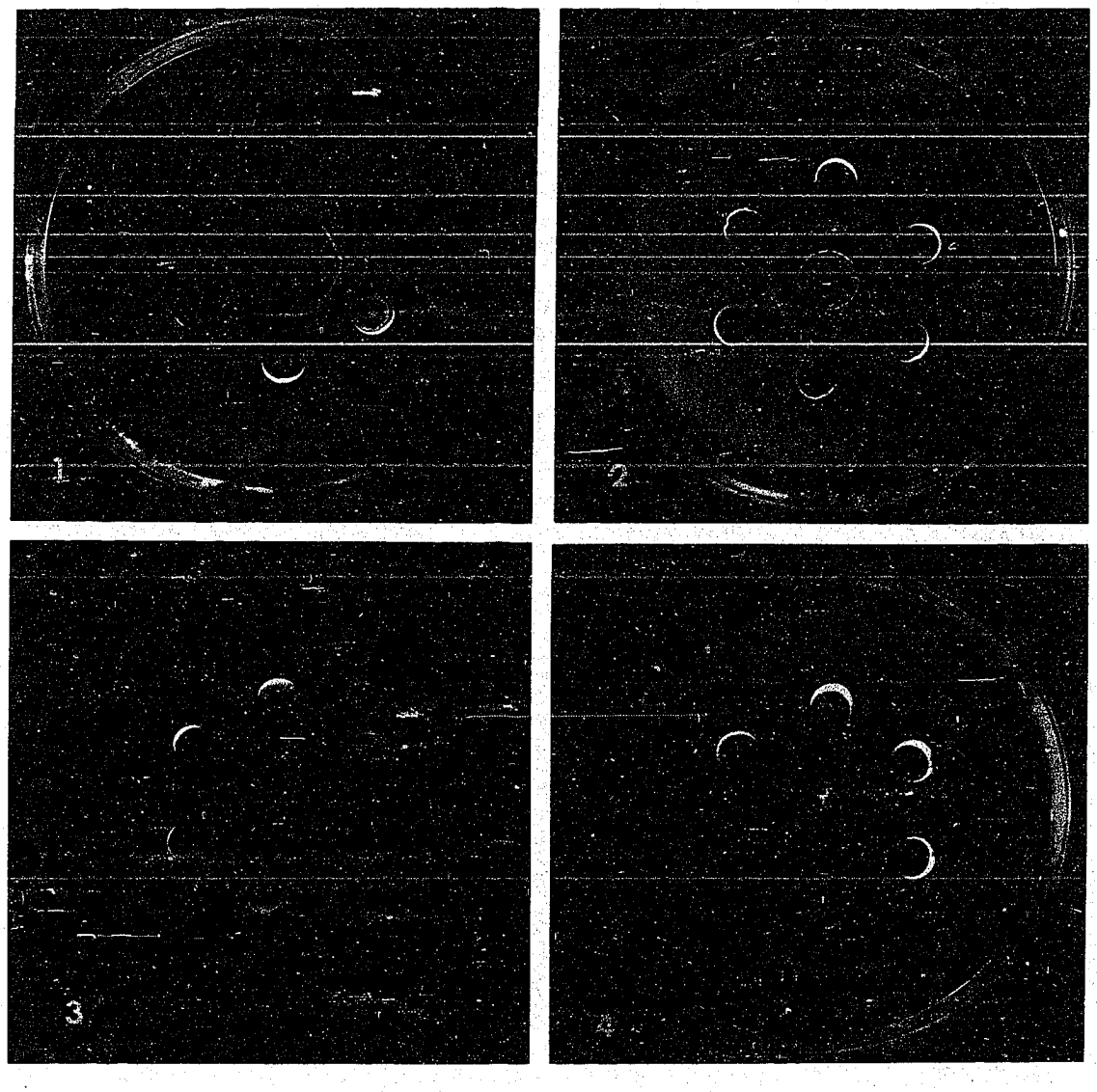

1
2
3
4

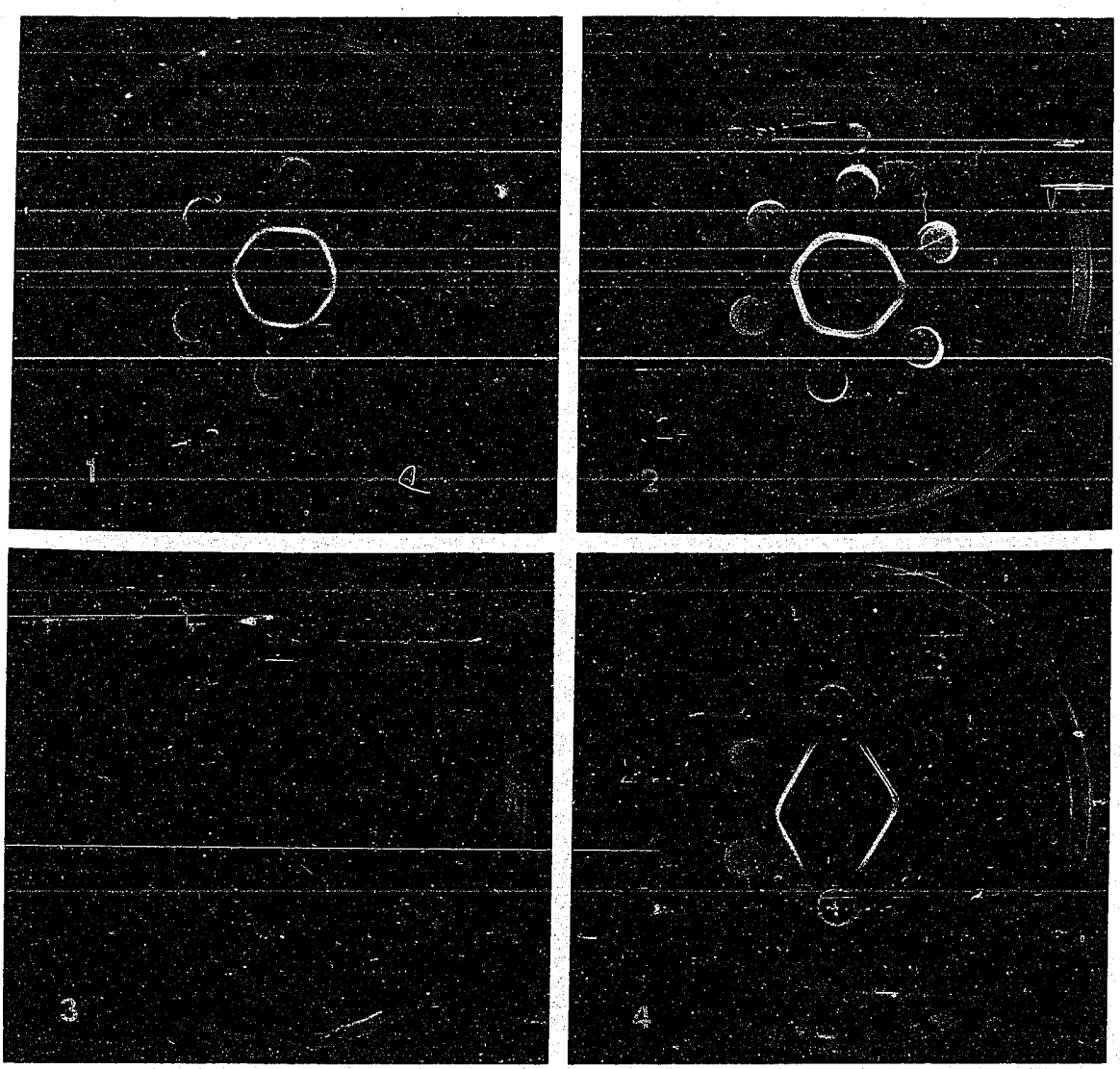
Figure 11. Photographs of representative gel diffusion plates showing reactions of antigens of scutellum, embryo and root from various varieties with Cherokee antiserum. Antiserum placed in center well and variety extracts in the outer wells. (letters near the wells designate extract, A = Andrew, C = Cherokee, M = Mo. 0-205, N = Nemaha)

Plate 1. Scutellum extracts and antiserum

Plate 2. Embryo extracts and antiserum

Plate 3. Root extracts and antiserum

Plate 4. Embryo extracts and antiserum. This plate was designed to show the non-specific reaction, normal serum was placed in the upper and lower wells (unmarked wells)


1
2
3
4

All visible bands showed interference and no spurs. Therefore, at least the stronger antigens were common to all varieties.

For each variety only one band formed by the reaction of the antigens of its whole-grain extract against Cherokee antiserum (Plate 4 of Figure 10) and there was interference wherever the bands met indicating that the apparent antigen was common to all four varieties.

The reaction of scutellum variety extracts (Plate 1 of Figure 11) was manifested by a broad diffuse band made up of several poorly separated bands. As with pollen, the actual plates showed better resolution and as many as four bands could be distinguished, no spurs were observed indicating antigens were in common. No qualitative differences were observed.

Plate 2 of Figure 11 shows the embryo variety extracts against Cherokee embryo antiserum. Four bands were produced by each variety and no spurs were visable at the junction zone. Again, the antigens contributing the major portion of the reaction were in common to all varieties.

The variety extracts of roots each produced two bands showing interference in the junction zone (Plate 3 in Figure 11), indicating antigens in common. Plate 4 of Figure 11 was designed to demonstrate the non-specific reaction present in the quantitative embryo tube tests. Normal serum was placed in the unmarked wells at top and bottom of the plate. A non-

specific reaction should deposit a band between the normal serum and the antigen. The absence of a band in this area indicated that either a non-specific reaction did not occur or that the non-specific reaction was not expressed in the same manner as an antibody-antigen type reaction.

The gel-diffusion plates showed similar reaction patterns for each plant part. One protein band occurred with whole-grain antigen-antibody reactions and as many as five bands occurred with coleoptile extracts. Therefore, between one and five specific reactions were detectable, depending on the plant part assayed. However, any reaction that was discernable for a plant part was common to all varieties. Of course, qualitatively different antigens could exist among varieties, but the antigens responsible for the major portion of the reaction were common to all varieties tested.

Comparisons of plant part antigens

The relationships of antigens from different plant parts are shown in Figures 12 and 13. Shown in Plate 1 in Figure 12 are the six plant part extracts--pollen, coleoptile, scutellum, whole-grain, root and embryo--against pollen antiserum (all for Cherokee variety). The pollen extract showed a strong and the root extract a very weak reaction indicating some homology between pollen and root antigens. The other extracts did not react with the pollen antiserum. Embryo, scutellum and whole-grain extracts showed reactions with embryo

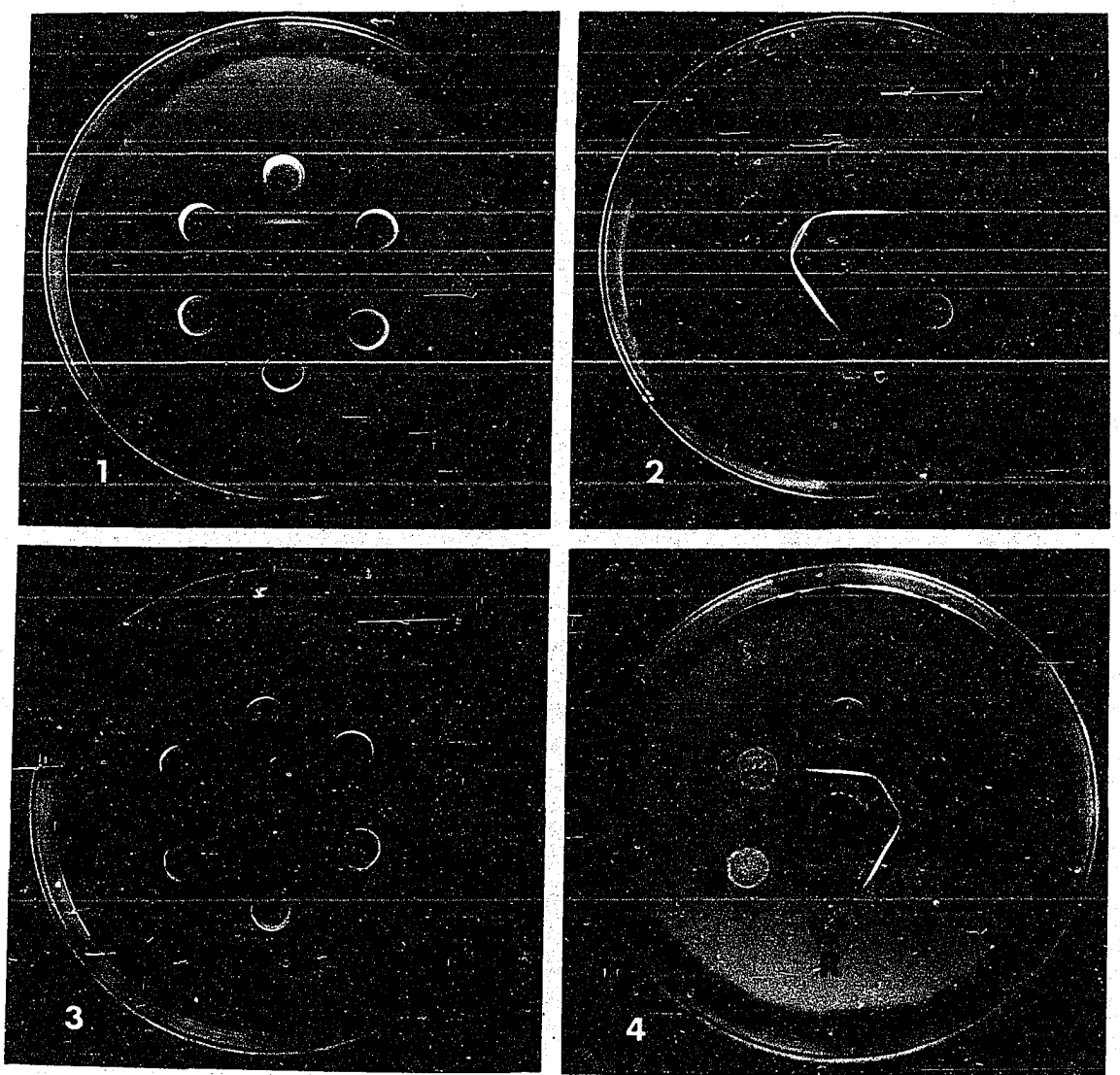
Figure 12. Photographs of representative gel diffusion plates showing reactions of Cherokee plant part antigens with Cherokee pollen, embryo, whole-grain and scutellum antisera. Antiserum placed in center well and plant part extracts in the surrounding wells. (Letters designate plant part extract, P = pollen, E = embryo, G = whole-grain, S = scutellum, R = root and C = coleoptile)

Plate 1. Plant part extracts and pollen antiserum

Plate 2. Plant part extracts and embryo antiserum

Plate 3. Plant part extracts and whole-grain antiserum

Plate 4. Plant part extracts and scutellum antiserum


1
2
3
4

antiserum, and each produced a strong band consisting of at least two components (Plate 2 of Figure 12). The interaction of these bands at the junction zone showed they were identical in the three extracts. Pollen, coleoptile and root extracts did not react. Antigens from whole-grain, embryo and scutellum reacted with whole-grain antiserum, so these three plant parts contain antigens in common. Embryo and scutellum produced more precipitate than whole-grain. Antigens from scutellum, whole-grain and embryo reacted with scutellum antiserum (Plate 4 of Figure 12). In each reaction only one band was evident and interaction existed where the bands from different extracts met indicating identical antigens in these three plant parts.

Plate 1 in Figure 13 shows root, pollen, coleoptile and embryo each produced a band with root antiserum, interference at the junction indicates an identical antigen in these plant parts. In addition, root extract produced a second band not observed with the other extracts indicating the presence of an antigen exclusive to root. Whole grain and scutellum antigens did not produce reactions. Plate 2 shows coleoptile antiserum reacted with coleoptile antiserum only, suggesting the presence of an antigen exclusive to coleoptile.

Table 3 summarizes the relationships of the plant part antigens. Pollen antigens have some homology with root antigens; the grain parts and whole-grain have common antigens. Both coleoptile and embryo antigens have some homology with root antigens.

Figure 13. Photographs of representative gel diffusion plates showing reactions of Cherokee antigens of the plant parts with Cherokee root and coleoptile antisera. Antiserum placed in center well and plant part extracts in the surrounding wells. (Letters designate plant part extract, P = pollen, E = embryo, G = whole-grain, S = scutellum, R = root and C = coleoptile)

Plate 1. Plant part extracts and root antiserum

Plate 2. Plant part extracts and coleoptile antiserum

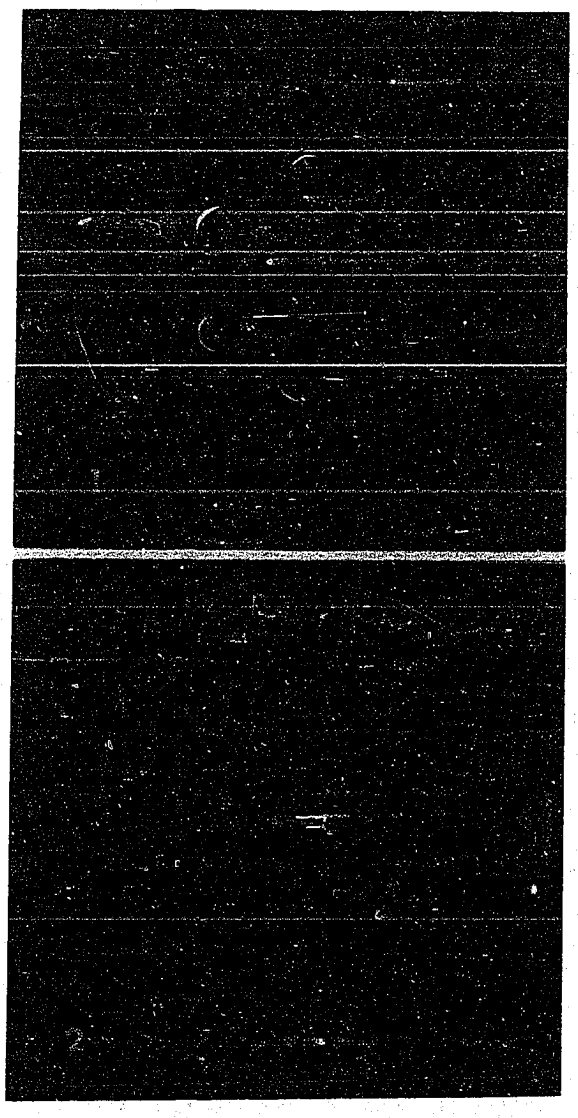


Table 3. Summary of plant part antigen relationships from gel diffusion tests ( + + = homology, + = partial homology)

| Antiserum | Antigen | | | | | |
|---|---|---|---|---|---|---|
| | Pollen | Embryo | Whole-grain | Scutellum | Root | Coleoptile |
| Pollen | + + | | | | + | |
| Embryo | | + + | + + | + + | | |
| Whole-grain | | + + | + + | + + | | |
| Scutellum | | + + | + + | + + | | |
| Root | + | + | | | + + | + |
| Coleoptile | | | | | | + + |

# DISCUSSION

Since the protein complex of a genotype is controlled by DNA and mediated through RNA it is reasonable that an index of genetic relationship can be obtained by comparing the proteins of different genotypes. Closely related genotypes should have similar proteins and those more distantly related should have more diversity between their protein complexes. On this assumption it is possible to construct a model that explains how this diversity or similarity could be measured serologically. A simplied form of such a model is given in Figure 14. Varieties 1 and 2, and 2 and 3 have two proteins in common and varieties 1 and 3 have only one in common, so they have a decreasing order of genetic relationship. When extracts of these varieties are injected into animals they should stimulate the production of antibodies against each of the proteins contained in the extract, e.g., animals injected with extract from Variety 1 should produce anti-A, -B, and -C, and with an extract from Variety 3, anti-A, -E, and -F.

If antiserum of Variety 1 is used to titrate the antigen extracts of the three varieties, Variety 1 should produce the greatest reaction because it is homologous with the antiserum. For discussion purposes the reaction is assumed to be 100 units. Variety 2 should produce a lower reaction than Variety 1, but a greater reaction than Variety 3. Assume these reactions are

75 and 50 units, respectively. Thus we expect reactions of decreasing magnitude as varieties with greater protein dissimilarities are tested.

Similarly, if the antiserum against Variety 3 is used to titrate the antigen extracts of the three varieties (the reciprocal comparison) antigen from Variety 3 should produce a reaction of 100 units, and antigen extracts from Varieties 2 and 1 should produce 75 and 50 units, respectively.

If the percent of serological relationship of Variety 1 to Variety 3 is calculated by the equation--Heterologous reaction ÷ Homologous reaction x 100--the relationship would be 50% and both methods (reciprocal comparisons) would produce the same serological value. The mechanism in this model applies when, (a) the homologies of antigens and antibodies govern the magnitude of the reaction, (b) values from reciprocal comparisons agree, and (c) no heterologous reaction exceeds the homologous reaction in magnitude. Qualitative antigen differences would be measured collectively in a quantitative manner.

The mechanism and limitations of reaction in Model 1 would be the ideal if serology is to be used successfully to assay genetic relationships of varieties. However, quantitative serological data herein do not fit this model. Two discrepancies exist, (1) heterologous reactions were often greater than the corresponding homologous reaction and (2) readings

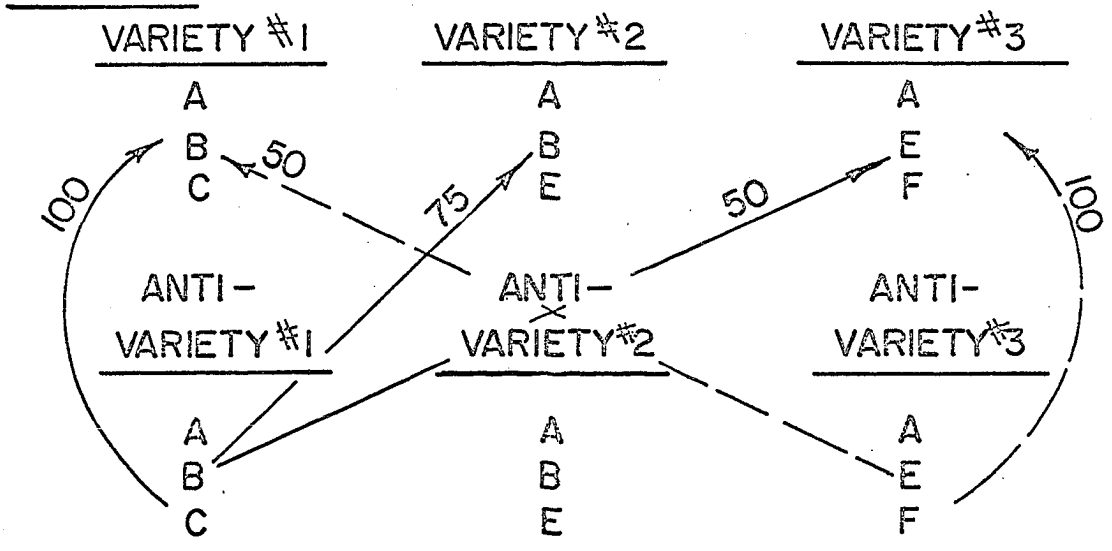
from reciprocal comparisons did not agree, and in fact, generally, when the relationship of two varieties was lower than 100 by one method it was higher than 100 by the reciprocal method. These discrepancies were consistent for all plant parts tested and can be illustrated as follows. If Cherokee antigen produced a reaction greater than Mo. 0-205 antigen with both Cherokee antiserum (Method A) and with Mo. 0-205 antiserum (Method B) the serological relationship of Mo. 0-205 to Cherokee measured by Method A would be less than 100, but that measured by Method B would be greater than 100. This is due to the method of calculation of the serological relationship (Heterologous reaction ÷ Homologous reaction x 100). With Method A the greater Cherokee reaction is the denominator giving a relationship less than 100, and with Method B the greater Cherokee reaction is the numerator giving a relationship greater than 100.

Model 2, presented in Figure 15, can account for the readings obtained herein. In this model the three varieties are represented by a common antigen which could be the predominant antigen in all varieties. Variety 1 has the greatest quantity of Antigen A, followed by Variety 2 with a lesser amount and Variety 3 with the least. When extracts of these varieties are injected into animals each produces anti-A. The animal injected with antigen extract from Variety 1 may produce an antiserum of higher titer than the one receiving Variety 3 extract.

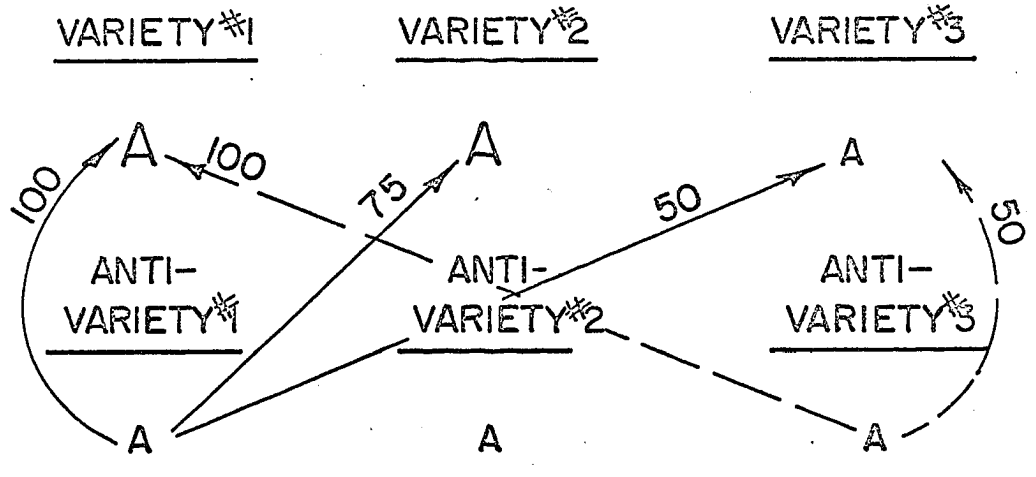
Figure 14. Illustration of mechanism that depends upon the homologies of the antigen and antiserum for the serological differences

Figure 15. Illustration of mechanism that expresses differences due only to antigen quantity

MODEL 1




MODEL 2



Animal differences could also be involved, but these differences would be unimportant because the antisera are adjusted to an equivalent reacting capacity (as nearly as possible) before being used in the in vitro tests and antigen sources are assigned to guinea pig replicates at random. The antisera can therefore be considered equivalent from all three varieties.

When antiserum of Variety 1 is used to titrate the antigens of the three varieties, Variety 1 antigen will give the greatest reaction, i.e., 100, Variety 2 next greatest, 75, and Variety 3 the least, 50. The serological relationships of Variety 2 and Variety 3 to Variety 1 will be 75 and 50%, respectively (Heterologous reaction ÷ Homologous reaction x 100). With reciprocal titrations using Variety 3 antiserum, the reaction with Variety 1 will still be largest, 100, and Variety 3 will still be the smallest, 50. Since the homologous reaction is less than the heterologous reaction, the serological relationship of Variety 3 to Variety 1 will be 200 (Heterologous reaction ÷ Homologous reaction x 100).

This model can account for (1) serological relationships over 100 and (2) the reciprocal results from Method A and Method B. The serological relationships for this mechanism are due solely to the quantitative differences of common antigens in all varieties. Homologies between antigen and antisera do not contribute to these serological relationships, and consequently, differences due to this mechanism have little value

for predicting genetic relationship.

The combination of Models 1 and 2 would be a model that when extended should account for all of the serological differences between two organisms. Model 1 would account for the differences between distantly related individuals and the closer the relationship between individuals the more important Model 2 would become because more and more antigens would be in common.

Even though the quantitative data and the gel diffusion tests in this study suggest that Model 2 (Mechanism 2) was operating, Model 1 (Mechanism 1) may also be operative to some degree. However, its effects were masked by the differences due to common antigens (Mechanism 2). If the effects of Mechanism 1 could be separated from those of Mechanism 2, they could be used in predicting the genetic relationships among varieties.

The fact that Mechanism 1 produces ratios (Heterologous reactions ÷ Homologous reactions) less than 1 with both Methods A and B, and that Mechanism 2 produces ratios reciprocal to one another with Method A and B, should be useful in determining whether Mechanism 1 is operative and should also give insight into the relationships of the varieties.

Assume all of the serological differences between two varieties are due to Mechanism 2 and that Methods A and B are comparable so their ratios are reciprocal to one another.

Multiplication of the ratio of Method A with that of Method B would give a product of one, and extraction of the square root would not change this value. This would cancel the differences due to Mechanism 2, the overall effect would be similar to antigens in common, yet quantitatively equivalent, and would not contribute to the serological relationships. Again assume that all of the differences are due to Mechanism 1 and that Methods A and B are comparable so that the ratios are below one and are equal for both methods. Treatment similar to the above would give a product equal to the square of either ratio. The extraction of the square root of this product would restore the ratio to its original value, less than one, depending on the homologies of the antigens and antisera.

If the assumption that Methods A and B are comparable is met, the following equation should indicate whether Mechanism 1 is operative and give indications of genetic relationships.

$$\sqrt{\frac{\text{Heterologous turbidity}_A}{\text{Homologous turbidity}_A} \times \frac{\text{Heterologous turbidity}_B}{\text{Homologous turbidity}_B}} \times 100 =$$

Adjusted Serological Relationship

A = Method A

B = Method B

Although the adjusted serological relationships are not unbiased estimates of Mechanism 1, relationships less than 100

should be due to the qualitative differences of Mechanism 1.

This type of data summarization was not anticipated at the beginning of this study, but attempts were made to make the reciprocal comparisons as comparable as possible. Standard procedures were used to produce antisera, the most uniform antisera were selected and these were adjusted to a more uniform precipitating capacity. The same antigen extract was used for the reciprocal tests and the physical conditions were kept uniform.

Table 4 shows the adjusted serological relationships of the varieties to Cherokee when calculated as described above. The varieties are in descending order of relationship based on the grain yield variance components. The adjusted serological relationships should place the varieties in this same order.

The adjusted pollen serological relationships are all nearly the same. The relationship of Minland to Cherokee at 102% was high because the second Minland antisera was unreasonably high. The adjusted coleoptile serological relationships did not agree with expectation because the relationship of Andrew to Cherokee was lower than that of Mo. 0-205. The close relationship of Nemaha to Cherokee was expected. The whole-grain relationships agreed well with expectation.

The adjusted serological relationships of scutellum and embryo were not reasonable because relationships above 100%

are not possible. The antigens of these two plant parts also produced large non-specific reactions. The adjusted serological relationships of embryo from reciprocal comparisons of dilutions of antiserum titrated by a constant antigen of a low enough concentration to avoid the non-specific reactions, agreed with expectation. Although these data were limited--only relationships of two varieties to Cherokee--they support the idea that the unreasonable values for scutellum and embryo (Table 4) were due to non-specific reactions. On this basis the unreasonable scutellum and embryo relationships will not be discussed further.

The adjusted serological relationships among varieties based on pollen differed from coleoptile and these differed from those of whole-grain and embryo. Only those of whole-grain and embryo agreed with the relationships estimated by grain yield variance components. It is interesting to note that (of these plant parts) only whole-grain and embryo have antigens in common according to the gel diffusion tests (Figures 12 and 13). Probably the best overall serological prediction of genetic relationship is the mean of the adjusted serological relationships of all plant parts. Means of pollen, coleoptile, whole-grain, and embryo (Table 4) show good agreement with the grain yield variance components.

Mechanism 2 contributes unwanted reactions to serological relationships, but no unbiased method exists whereby its con-

Table 4. Adjusted serological relationships of the oat varieties to Cherokee calculated as described on pages 82 and 83 of the text (varieties listed in descending order of relationship based on the grain yield variance components)

| Variety | Pollen | Coleoptile | Whole-grain | Scutellum[a] | Embryo[b] | Embryo[c] | Average[d] |
|---|---|---|---|---|---|---|---|
| Nemaha | 97 | 99 | 97 | 101 | 135 | -- | 97.6 |
| Bonham | 97 | | | | | | |
| Richland | 96 | | | | | | |
| Andrew | 98 | 90 | 96 | 107 | 145 | 98 | 95.5 |
| Minland | 102 | | | | | | |
| Mo. 0-205 | 97 | 95 | 90 | 101 | 144 | 88 | 92.5 |

[a]Antigen produced non-specific reactions.

[b]Antigen produced non-specific reactions (higher).

[c]Calculated from comparisons using dilutions of antisera titrated with constant antigen which had low enough concentrations to avoid non-specific reactions (based on only one set of comparisons).

[d]Includes only pollen, coleoptile, whole-grain and embryo.

founding effects can be completely removed from the data. Therefore, serological techniques should be modified to minimize the effects of Mechanism 2 and amplify those differences due to Mechanism 1. Several factors contribute to the effects of Mechanism 2. First, there are quantitative differences in the antigen concentration of the extracts. Protein concentration of the extracts can be standardized by using any one of a number of chemical procedures, but these tests measure protein concentration on the basis of different parts of the protein molecule, and thus, do not give equivalent readings with different kinds of proteins. Second, there is no assurance that the active antigens are measured with equivalent precision. The antigen concentrations of these extracts can be standardized serologically, and this in fact, is done when the complete reaction range of the antigens are titrated with a single antiserum.

Differences in antigen concentration cause the titration curves to shift in position either to the right or left, but do not change the area under the curve (total reaction). Serological standardization should give more accurate results than chemical methods because it is based on the active antigens of the extracts. Although this procedure works for single antigens, the effects of Mechanism 2 are not completely removed when mixtures of antigens, such as those of a plant extract, are titrated. The pollen data herein and that of Kleese

(1962) give essentially the complete reaction range, and yet the data show considerable confounding due to Mechanism 2.

Non-specific reactions contribute directly to the effects of Mechanism 2, and in addition, as evidenced from the quantitative embryo and scutellum data, are not consistent. Thus, it is important to avoid non-specific reactions. Hammond (1952 and 1955), Baum (1954) and Johnson (1954) using this quantitative serological technique to study plant relationships, employed partially purified plant proteins to avoid non-specific reactions. Rohringer and Stahmann (1958) avoided non-specific reactions of crude extracts of tomato leaves by fractionating the antiserum with alcohol and removing the fraction of the antiserum that caused the non-specific reaction.

I used crude extracts because it seemed that they best represented the complete protein complement of the plant, and that fractionation procedures would remove some important proteins. These crude extracts caused many problems both in the in vitro tests as already mentioned, and in the immunization of animals. They contain metabolic products that are often toxic to animals. As an example, when guinea pigs were immunized with crude extracts of coleoptile and root by intraperitonaeal injections, many animals died before titer was observed and the method had to be abandoned. The problems presented by crude extracts probably more than offset their advantages.

If the common antigens could be removed, the remaining serological differences would be due to Mechanism 1 and would be valuable in predicting genetic relationships. Absorption techniques could be used to eliminate most or all of these reactions. These have been used successfully in plant systematics by Nelson and Birkeland (1929) working with wheat, by Gell et al. (1959) working with species within the genus Solanum, and by Kloz et al. working with beans.

The data of this study provide evidence that serology can predict genetic relationships among oat varieties, but the technique must be refined to minimize the problems which have been mentioned.

## SUMMARY

The serological relationships among several oat varieties were assayed using antigens from pollen, coleoptile, whole-grain, scutellum and embryo. The relationships of six varieties to Cherokee were determined using pollen antigens and relationships of three varieties to Cherokee were made using the other plant part antigens. The serological relationships were determined by comparing photoelectrically measured turbidities of heterologous precipitin reactions to those of the homologous reactions. Reciprocal comparisons were made using Cherokee antisera vs. the variety antigens (Method A) and the variety antisera vs. the homologous and Cherokee antigens (Method B). The serological relationships were compared to the genetic relationships estimated by the within cross grain yield variance components. Gel diffusion comparisons were also made to study the qualitative antigen differences among the oat varieties for each plant part and among the different plant parts.

The serological relationships of the varieties determined by the two methods were not in agreement. Estimates of relationship produced by Method A generally agreed with the genetic relationships estimated by the grain yield variance components, whereas Method B generally gave relationships above 100% and reciprocal to Method A. Since theoretically

both methods are equally valid for measuring genetic relationships, Method A could not be selected in preference to Method B.

Models of mechanisms to account for the observed relationships were proposed. The quantitative tests and gel diffusion data supported the hypothesis that a mechanism due to quantitative differences of antigens common to all varieties (Mechanism 2) was active and that its effects masked the differences due to homology differences of the antibodies and antigens (Mechanism 1). Since quantitative differences due to common antigens were not valuable in predicting genetic relationships they confounded the data.

A system of data summarization was proposed which showed that Mechanism 1 was also operative. Although unbiased serological estimates of genetic relationship were not available, these data indicated that whole-grain and embryo predicted genetic relationship most similar to those estimated by the grain yield variance components.

Gel diffusion tests showed that the oat variety extracts contained antigens in common, but no qualitative differences were evident. Other gel diffusion tests showed that whole-grain, embryo and scutellum contained antigens in common and that some homologies existed between root, pollen, coleoptile and embryo antigens.

Suggestions were made for modifying the technique to avoid the confounding effects of common antigens and unwanted non-specific reactions.

# LITERATURE CITED

Avery, Oswald T., MacLeod, Colin M. and McCarty, Maclyn. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Journal of Experimental Medicine 79: 137-158.

Baum, W. C. October, 1954. Systematic serology of the family Cucurbitaceae with special reference to the genus Cucurbita. Serological Museum Bulletin No. 13: 5-8.

Beadle, George W. and Tatum, Edward L. 1941. Genetic control of biochemical reactions in Neurospora. Proceedings of National Academy of Sciences 27: 499-506.

Bordet, Jules. 1899. LeMecanisme de l'aggultination. Annales Institute Pasteur 13: 225.

Boyden, Alan. 1954. Measurement and significance of serological correspondence among proteins. Rutgers University Bureau of Biological Research 10: 74-97.

Boyden, Alan, Bolton, E. and Gemeroy, D. 1947. Precipitin testing with special reference to the photoelectric measurement of turbidity. Journal of Immunology 57: 211-227.

Boyden, Alan and DeFalco, Ralph. 1943. Report on use of the photronreflectometer in serological comparisons. Physiological Zoology 16: 130-141.

Bremer, Hans and Konrad, Michael W. 1964. A complex of enzymatically synthesized RNA and template DNA. Proceedings of National Academy of Sciences 51: 801-808.

Chester, K. Starr. 1937. A critique of plant serology. II. Application of serology to the classification of plants and the identification of plant products. Quarterly Review of Biology 12: 165-190.

Davidson, J. F. and Thompson, T. L. 1956. Phytoserology versus genealogy in Zea mays. Madrono 13: 252-259.

Dawson, M. H. and Sia, R. H. P. 1931. In vitro transformation of pneumococcal types. Journal of Experimental Medicine 54: 681-710.

Exposito, V. M., Ulrich, V. and Burrell, R. G. 1966. A serological study of Medicago sativa L. varieties. Crop Science 6: 489-491.

Fairbrothers, David E. and Johnson, Marion A. 1959. The precipitin reaction as an indicator of relationship in some grasses. Recent Advances in Botany 9: 117-119.

Fraenkel-Conrat, H. and Singer, B. 1957. Virus reconstitution. II. Combination of protein and nucleic acid from different strains. Biochima et Biophysica Acta 24: 540-548.

Freund, J., Thomson, K. J., Hough, H. B., Sommer, H. E., and Pisani, T. M. 1948. Antibody formation and sensitization with the aid of adjuvants. J. Immunol. 60: 383-398.

Gardner, Eldon J. c1960. Principles of genetics. John Wiley and Sons, Inc. New York, N.Y.

Garrod, A. E. 1909. Unborn errors of metabolism. Oxford University Press. London. Original not available for examination; cited in Srb, A. M., Owen, R. D. and Edgar, R. S. c1965. General genetics. 2nd. ed. W. H. Freeman and Co. San Francisco, Calif.

Gell, P. G. H., Hawkes, J. G. and Wright, S. T. C. 1959. The application of immunological methods to the taxonomy of species within the genus Solanum. Proceedings of Royal Society of London Series B, 151: 364-383.

Gilg, E. and Schürhoff, P. H. 1927. Unsere Erfahrungen über die Brauchbarkeit der Serodiagnostik für die Botanische Verwandtschaftsforschung. Berlin Deutsche Botanische Gesellschaft 45: 315-330.

Griffith, Fred. 1928. The significance of pneumococcal types. Journal of Hygiene 27: 113-159.

Hall, Ove. 1959. Immuno-electrophoresis analysis of allopolyploid rye-wheat and its parental species. Hereditas 45: 495-504.

Hammond, H. David. 1952. Serology applied to systematic studies in the Ranunculaceae. Unpublished Ph.D. thesis. Library, University of Pennsylvania, Philadelphia, Pa.

Hammond, H. David. May, 1955. A study of taxonomic relationship within the Solanaceae as revealed by the photron'er serological method. The Serological Museum Bulletin No. 14: 3-5.

Hayashi, Masaki, and Spiegelman, S. 1961. The selective synthesis of informational RNA in bacteria. Proceedings of National Academy of Science 47: 1564-1580.

Hershey, A. D. and Chase, Martha. 1952. Independent functions of viral protein and nucleic acid in the growth of bacteriophage. Journal of General Physiology 36: 39-56.

Horowitz, N. H. and Leupold, U. 1951. The one gene - one enzyme hypothesis. Cold Spring Harbor Symposia of Quantitative Biology 16: 65-74.

Ingram, V. M. 1957. Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin. Nature 180: 326-328.

Johnson, M. A. October, 1954. The precipitin reaction as an index of relationships in the Magnoliaceae. Serological Museum Bulletin No. 13: 1-5.

Kleese, Roger Allen. 1962. Serological predictions of genetic relationships among oat varieties and corn inbreds. Ph.D. thesis. Library, Iowa State University of Science and Technology. Ames, Iowa.

Kleese, R. A. and Frey, K. J. 1964. Serological predictions of genetic relationships among oat varieties (Avena sativa L.) and corn inbreds (Zea mays L.). Crop Science 4: 379-383.

Kloz, J., Turkova, V. and Klozova, E. 1959. Serological investigation of taxonic specificity of proteins in various plant organs in some taxons of the family Viciaceae. Biologia Plantarum 2: 126-137.

Kowarski, Alb. 1901. Uber den Nachweis von Pflanzlichem Eiweiss auf Biologischen Wege. Deutsche Medizinische Wochenschrift 27: 442.

Kraus, Rudolf. 1897. Ueber Specifische Reaktionen in Keimfreien Filtraten aus Cholera, Typhus, und Pestbouillonculturen, Erzeugt Durch Homologes Serum. Wiener klinische Wochenschrift 10: 736.

Landsteiner, Karl. 1946. The specificity of serological reactions. Revised edition. Harvard University Press. Cambridge, Mass.

Langridge, J. and Brock, R. D. 1961. A thiamine-requiring mutant of the tomato. Australian Journal of Biological Sciences 14: 66-69.

Leone, C. A. 1952. Effect of multiple injections upon specificity of antisera. Journal of Immunology 69: 285-295.

Libby, Raymond L. 1938. The photronreflectometer - an instrument for the measurement of turbid systems. Journal of Immunology 34: 71-73.

Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. 1958. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265.

McFarland, J. 1907. The nephlometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the American Medical Association 49: 1176-1178.

Myers, Walter, Cantab, M. B. and Walker, John L. 1900. On immunity against proteids. Lancet 2: 98-100.

Nelson, C. I. and Birkeland, J. M. 1929. A serological ranking of some wheat hybrids as an aid in selecting certain genetic characters. Journal of Agricultural Research 38: 169-181.

Nirenberg, N. W. and Matthaei, J. H. 1961. The dependence of cell-free protein synthesis in Escherishia coli upon naturally occurring or synthetic polyribonucleotides. Proceedings of National Academy of Sciences 47: 1588-1602.

Nuttall, George H. F. 1901. Experiments upon the new specific test for blood. British Medical Journal 1: 1141.

Nuttall, George H. F. 1904. Blood immunity and blood relationship. Cambridge University Press. London, England.

Ouchterlony, O. 1948. In vitro method for testing the toxin-producing capacity of diphtheria bacteria. Acta Pathologica et Microbiologica Scandinavica 25: 186-191.

Ouchterlony, O. 1949a. Antigen - antibody reactions in gels. II. Factors determining the site of the precipitate. Arkiv for Kemi 1: 43-48.

Ouchterlony, O. 1949b. Antigen - antibody reactions in gels. III. The time factor. Arkiv for Kemi 1: 55-59.

Ouchterlony, O. 1958. Diffusion-in-gel methods for immunological analysis. Progress in Allergy 5: 1-78.

Rohringer, R. and Stahmann, M. A. 1958. Immunochemical studies with tomato leaf proteins. Science 127: 1336-1337.

Schroeder, Walter A. 1963. The hemoglobins. Annual Review of Biochemistry 32: 301-320.

Schwartz, Drew. 1960. Electrophoretic and immunochemical studies with endosperm proteins of maize mutants. Genetics 45: 1419-1427.

Spiegelman, S., Hall, Benjamin D. and Storck, R. 1961. The occurrence of natural DNA-RNA complexes in Escherichia coli infected with $T_2$. Proceedings of National Academy of Science 47: 1135-1141.

Srb, Adrian M., Owen, Ray D. and Edgar, Robert S. c1965. General genetics. 2nd ed. W. H. Freeman and Co. San Francisco, Calif.

Stadler, L. J. and Uber, Fred M. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics 27: 84-118.

Sturtevant, A. H. and Beadle, G. W. 1939. An introduction to genetics. W. B. Saunders Co. Philadelphia, Pa.

Uhlenhuth, P. 1900. Neuer Beitrag zum specifischen Nachweis bon Eiereiweiss auf Biologischen Wege. Deutsche Medicinische Wochenschrift 26: 734-735.

Umbarger, H. Edwin and Mueller, J. Howard. 1951. Isoleucine and valine metabolism of Escherichia coli. Journal of Biological Chemistry 189: 277-285.

Urano, K. 1955. Preliminary investigations on diagnosis of strain-relationship in corn plant by precipitine test. I. Precipitine reaction between inbred lines of corn. English Summary. Crop Science Society of Japan Proceedings 24: 4-7.

Urano, K. 1959. Serological investigation with the phylogenetical relationship among inbred lines in maize. Maize Genetics Cooperation News Letter 33: 82-84.

Watson, J. D. 1963. Involvement of RNA in the synthesis of proteins. Science 140: 17-26.

Watson, J. D. and Crick, F. H. C. 1953a. The structure of DNA. Cold Spring Harbor Symposia of Quantitative Biology 18: 123-131.

Watson, J. D. and Crick, F. H. C. 1953b. A structure for deoxyribose nucleic acids. Nature 171: 737-738.

Watson, J. D. and Crick, F. H. C. 1953c. Genetic implications of the structure of deoxyribose nucleic acid. Nature 171: 964-969.

Wilkins, M. H. F., Stokes, A. R. and Wilson, H. R. 1953. Molecular structure of deoxypentose nucleic acids. Nature 171: 738-740.

Wolf, H. R. 1935. The effect of species specificity of serum precipitins. Journal of Immunology 29: 1-11.

Yamasaki, Y., Suto, T. and Urano, K. 1957. Cytological and serological aspects of heterosis in maize. Proceedings of the International Genetics Symposium. 1956. Supplement Volume of Cytologia. pp. 396-400.

Zade, A. 1914. Serologisiche Studien an Leguminosen und Gramineen Zeitschrift fur Pflanzenzuchtung 2: 101-151.

Zamecnik, Paul C. 1959. Historical and current aspects of protein synthesis. Harvey Lectures Series 54: 256-281.

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. K. J. Frey for his assistance, guidance and patience during the course of this study. I am grateful for the advice given by Dr. I. C. Anderson, Dr. R. S. Allen, Dr. W. J. Miller and Dr. M. L. Kaeberle.

# A P P E N D I X

Table 5. Total turbidity of pollen serological reactions in galvanometer units, (McFarland's Nephlo Standard #1 = 100 units) of homologous and heterologous serological reactions using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Method A | | | | | |
| Cherokee | Cherokee | 76 | 89 | 85 | 47 |
| Cherokee | Andrew | 45 | 57 | 82 | 42 |
| Cherokee | Bonham | 43 | 59 | 69 | 54 |
| Cherokee | Minland | 33 | 38 | 75 | 44 |
| Cherokee | Mo. 0-205 | 75 | 88 | 99 | 59 |
| Cherokee | Nemaha | 40 | 64 | 69 | 42 |
| Cherokee | Richland | 53 | 54 | 82 | 44 |
| Method B | | | | | |
| Andrew | Andrew | 45 | 55 | | |
| Andrew | Cherokee | 52 | 74 | | |
| Bonham | Bonham | 53 | 44 | | |
| Bonham | Cherokee | 63 | 52 | | |
| Minland | Minland | 72 | 36 | | |
| Minland | Cherokee | 84 | 70 | | |
| Mo. 0-205 | Mo. 0-205 | 41 | 31 | | |
| Mo. 0-205 | Cherokee | 32 | 29 | | |
| Nemaha | Nemaha | 33 | 32 | | |
| Nemaha | Cherokee | 47 | 36 | | |
| Richland | Richland | 35 | 50 | | |
| Richland | Cherokee | 46 | 49 | | |

Table 6. Pollen serological relationships of six oat varieties to Cherokee expressed as percent (Heterologous turbidity ÷ Homologous turbidity x 100) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| Method A | | | | | | |
| Cherokee | Andrew | 59 | 64 | 96 | 89 | 77.0 |
| Cherokee | Bonham | 57 | 66 | 81 | 115 | 79.8 |
| Cherokee | Minland | 43 | 43 | 88 | 94 | 67.0 |
| Cherokee | Mo. 0-205 | 99 | 99 | 116 | 126 | 110.0 |
| Cherokee | Nemaha | 53 | 72 | 81 | 89 | 73.8 |
| Cherokee | Richland | 70 | 61 | 96 | 94 | 80.2 |
| Method B | | | | | | |
| Andrew | Cherokee | 116 | 135 | | | 125.5 |
| Bonham | Cherokee | 119 | 118 | | | 118.5 |
| Minland | Cherokee | 117 | 195 | | | 156.0 |
| Mo. 0-205 | Cherokee | 78 | 94 | | | 86.0 |
| Nemaha | Cherokee | 142 | 112 | | | 127.0 |
| Richland | Cherokee | 131 | 98 | | | 114.5 |

Table 7. Total turbidity of coleoptile serological reactions in galvanometer units (McFarland's Nephlo Standard #1 = 100 units) using guinea pig antisera

| Antisera | Antigen | Guinea Pig 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Method A | | | | | |
| Cherokee | Cherokee | 59 | 82 | 49 | 56 |
| Cherokee | Andrew | 40 | 62 | 29 | 41 |
| Cherokee | Mo. 0-205 | 42 | 77 | 48 | 50 |
| Cherokee | Nemaha | 53 | 77 | 41 | 47 |
| Method B | | | | | |
| Andrew | Andrew | 98 | 29 | | |
| Andrew | Cherokee | 99 | 39 | | |
| Mo. 0-205 | Mo. 0-205 | 69 | 44 | | |
| Mo. 0-205 | Cherokee | 64 | 50 | | |
| Nemaha | Nemaha | 66 | 61 | | |
| Nemaha | Cherokee | 68 | 73 | | |

Table 8. Coleoptile serological relationships of three oat varieties to Cherokee expressed as % relationship (Heterologous turbidity ÷ Homologous turbidity x 100) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| Method A | | | | | | |
| Cherokee | Andrew | 68 | 76 | 59 | 73 | 69.0 |
| Cherokee | Mo. 0-205 | 71 | 94 | 98 | 89 | 88.0 |
| Cherokee | Nemaha | 90 | 94 | 84 | 84 | 88.0 |
| Method B | | | | | | |
| Andrew | Cherokee | 101 | 135 | | | 118.0 |
| Mo. 0-205 | Cherokee | 93 | 114 | | | 103.5 |
| Nemaha | Cherokee | 103 | 120 | | | 111.5 |

Table 9. Total turbidity of whole grain serological reactions, in galvanometer units (McFarland's Nephlo Standard #1 = 100 units) of homologous and heterologous reactions using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Method A | | | | | |
| Cherokee | Cherokee | 34 | 25 | 49 | 55 |
| Cherokee | Andrew | 36 | 22 | 40 | 47 |
| Cherokee | Mo. 0-205 | 22 | 23 | 39 | 40 |
| Cherokee | Nemaha | 36 | 23 | 48 | 48 |
| Method B | | | | | |
| Andrew | Andrew | 25 | 49 | | |
| Andrew | Cherokee | 28 | 45 | | |
| Mo. 0-205 | Mo. 0-205 | 31 | 40 | | |
| Mo. 0-205 | Cherokee | 30 | 46 | | |
| Nemaha | Nemaha | 41 | 108 | | |
| Nemaha | Cherokee | 41 | 103 | | |

Table 10. Whole grain serological relationships of three oat varieties to Cherokee expressed as % relationship (Heterologous turbidity ÷ Homologous turbidity x 100) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| Method A | | | | | | |
| Cherokee | Andrew | 106 | 88 | 82 | 85 | 90.2 |
| Cherokee | Mo. 0-205 | 65 | 92 | 80 | 73 | 77.5 |
| Cherokee | Nemaha | 106 | 92 | 98 | 87 | 95.8 |
| Method B | | | | | | |
| Andrew | Cherokee | 112 | 92 | | | 102.0 |
| Mo. 0-205 | Cherokee | 97 | 115 | | | 106.0 |
| Nemaha | Cherokee | 100 | 95 | | | 97.5 |

Table 11. Total turbidity of scutellum serological reactions in galvanometer units (McFarland's Nephlo Standard #1 = 100 units) of homologous and heterologous serological reactions using guinea pig antiserum

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Method A | | | | | |
| Cherokee | Cherokee | 127 | 126 | 87 | 80 |
| Cherokee | Andrew | 127 | 95 | 62 | 77 |
| Cherokee | Mo. 0-205 | 113 | 90 | 67 | 70 |
| Cherokee | Nemaha | 133 | 124 | 75 | 85 |
| Method B | | | | | |
| Andrew | Andrew | 51 | 85 | | |
| Andrew | Cherokee | 69 | 113 | | |
| Mo. 0-205 | Mo. 0-205 | 86 | 84 | | |
| Mo. 0-205 | Cherokee | 103 | 109 | | |
| Nemaha | Nemaha | 76 | 81 | | |
| Nemaha | Cherokee | 74 | 90 | | |

Table 12. Scutellum serological relationships of three oat varieties to Cherokee expressed as % relationship (Heterologous turbidity ÷ Homologous turbidity x 100) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| Method A | | | | | | |
| Cherokee | Andrew | 100 | 75 | 71 | 96 | 85.5 |
| Cherokee | Mo. 0-205 | 89 | 71 | 77 | 88 | 81.2 |
| Cherokee | Nemaha | 105 | 98 | 86 | 106 | 98.8 |
| Method B | | | | | | |
| Andrew | Cherokee | 135 | 133 | | | 134.0 |
| Mo. 0-205 | Cherokee | 120 | 130 | | | 125.0 |
| Nemaha | Cherokee | 97 | 111 | | | 104.0 |

Table 13. Total turbidity of embryo serological reactions in galvanometer units (McFarland's Nephlo Standard #1 = 100 units) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Method A | | | | | |
| Cherokee | Cherokee | 83 | 63 | 32 | 63 |
| Cherokee | Andrew | 108 | 116 | 79 | 77 |
| Cherokee | Mo. 0-205 | 136 | 115 | 89 | 115 |
| Cherokee | Nemaha | 134 | 118 | 80 | 111 |
| Method B | | | | | |
| Andrew | Andrew | 116 | 66 | | |
| Andrew | Cherokee | 100 | 99 | | |
| Mo. 0-205 | Mo. 0-205 | 69 | 116 | | |
| Mo. 0-205 | Cherokee | 88 | 91 | | |
| Nemaha | Nemaha | 56 | 138 | | |
| Nemaha | Cherokee | 55 | 124 | | |

Table 14. Embryo serological relationships of three oat varieties to Cherokee expressed as % relationship (Heterologous turbidity ÷ Homologous turbidity x 100) using guinea pig antisera

| Antiserum | Antigen | Guinea Pig 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| Method A | | | | | | |
| Cherokee | Andrew | 130 | 184 | 247 | 122 | 170.8 |
| Cherokee | Mo. 0-205 | 164 | 183 | 278 | 182 | 201.8 |
| Cherokee | Nemaha | 161 | 187 | 250 | 176 | 193.5 |
| Method B | | | | | | |
| Andrew | Cherokee | 86 | 150 | | | 118.0 |
| Mo. 0-205 | Cherokee | 128 | 78 | | | 103.0 |
| Nemaha | Cherokee | 98 | 90 | | | 94.0 |

Table 15. Total turbidities of embryo titrations (dilutions of guinea pig antisera titrated by constant antigen ) in galvanometer units (McFarland's Nephlo Standard #1 = 100 units)

| Antisera | Antigen | Total Turbidity |
|---|---|---|
| Method A | | |
| Cherokee | Cherokee | 99 |
| Cherokee | Andrew | 79 |
| Cherokee | Mo. 0-205 | 66 |
| Cherokee | Nemaha | 99 |
| Method B | | |
| Andrew | Andrew | 42 |
| Andrew | Cherokee | 50 |
| Mo. 0-205 | Mo. 0-205 | 46 |
| Mo. 0-205 | Cherokee | 53 |

Table 16. Embryo serological relationships determined by titration dilutions of guinea pig antisera with constant antigen expressed as % relationship to Cherokee (Heterologous turbidities ÷ Homologous turbidities x 100)

| Antisera | Antigen | Relationship |
|---|---|---|
| Method A | | |
| Cherokee | Andrew | 80 |
| Cherokee | Mo. 0-205 | 67 |
| Cherokee | Nemaha | 100 |
| Method B | | |
| Andrew | Cherokee | 119 |
| Mo. 0-205 | Cherokee | 115 |

Table 17. Total turbidity of rabbit antisera titrations of pollen, whole-grain, and coleoptile antigens expressed in galvanometer units (McFarland's Nephlo Standard #1 = 100 units)

| Antisera | Antigens | Rabbit 1 | Rabbit 2 | Rabbit Av. |
|---|---|---|---|---|
| Pollen | | | | |
| Cherokee | Cherokee | 60 | | |
| Cherokee | Andrew | 56 | | |
| Cherokee | Bonham | 35 | | |
| Cherokee | Minland | 52 | | |
| Cherokee | Mo. 0-205 | 38 | | |
| Cherokee | Nemaha | 60 | | |
| Cherokee | Richland | 54 | | |
| Whole-Grain | | | | |
| Cherokee | Cherokee | 60 | 57 | 58.5 |
| Cherokee | Andrew | 63 | 54 | 58.5 |
| Cherokee | Mo. 0-205 | 59 | 61 | 60.0 |
| Cherokee | Nemaha | 65 | 58 | 61.5 |
| Coleoptile | | | | |
| Cherokee | Cherokee | 50 | | |
| Cherokee | Andrew | 54 | | |
| Cherokee | Mo. 0-205 | 58 | | |
| Cherokee | Nemaha | 48 | | |

Table 18. Serological relationships, using rabbit antisera, expressed as % relationship to Cherokee (Heterologous turbidity ÷ Homologous turbidity x 100)

| Antisera | Antigen | Rabbit 1 | Rabbit 2 | Rabbit Av. |
|---|---|---|---|---|
| Pollen | | | | |
| Cherokee | Andrew | 93 | | |
| Cherokee | Bonham | 58 | | |
| Cherokee | Minland | 87 | | |
| Cherokee | Mo. 0-205 | 63 | | |
| Cherokee | Nemaha | 100 | | |
| Cherokee | Richland | 90 | | |
| Whole-Grain | | | | |
| Cherokee | Andrew | 105 | 95 | 100 |
| Cherokee | Mo. 0-205 | 98 | 107 | 102 |
| Cherokee | Nemaha | 108 | 102 | 105 |
| Coleoptile | | | | |
| Cherokee | Andrew | 108 | | |
| Cherokee | Mo. 0-205 | 116 | | |
| Cherokee | Nemaha | 96 | | |

Figure 16. Typical titration curves of coleoptile antigens titrated with Cherokee coleoptile antisera. Initial antigen concentration 1:500

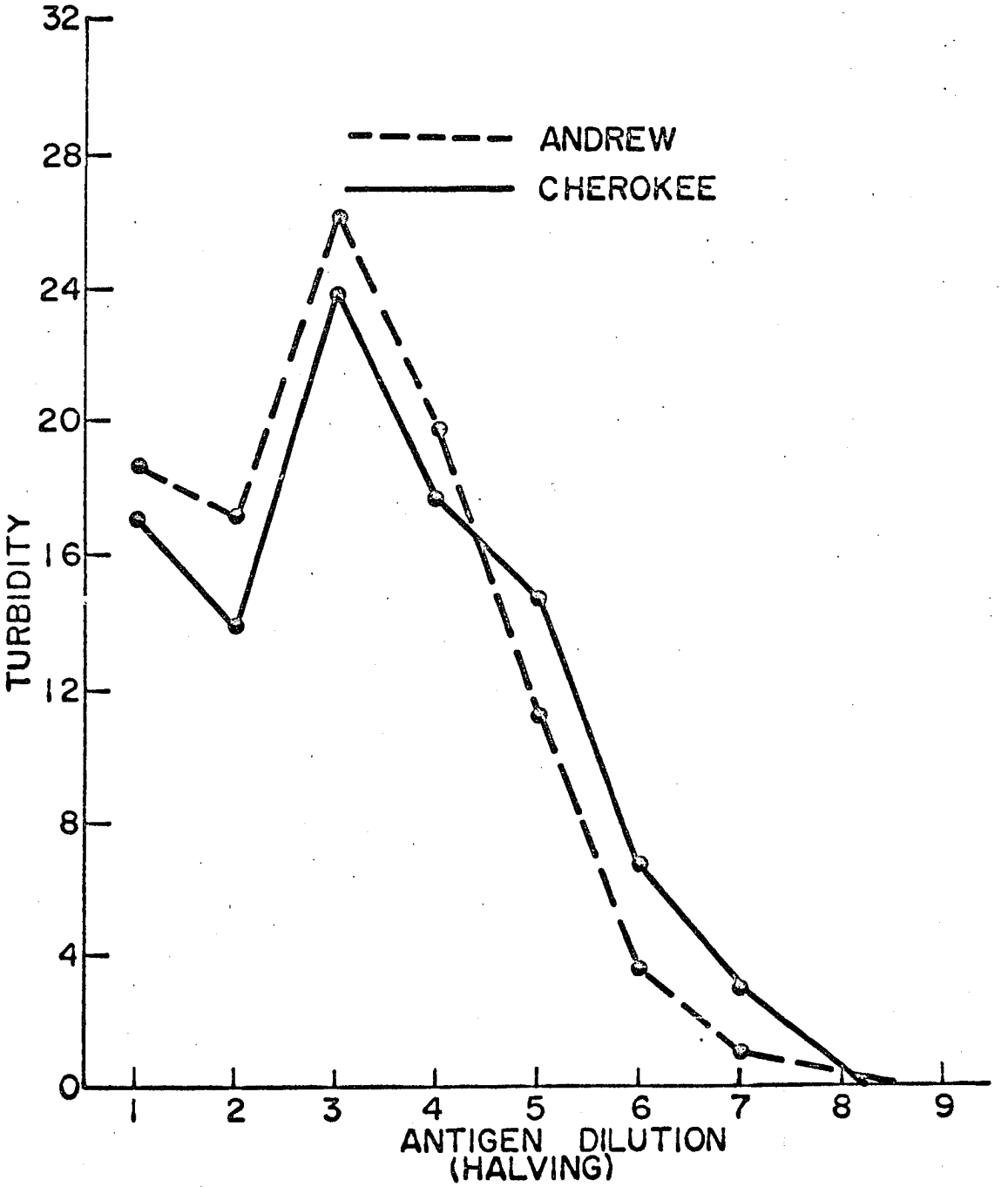

32
28
24
20
16
12
8
4
0
TURBIDITY
ANDREW
CHEROKEE
1
2
3
4
5
6
7
8
9
ANTIGEN DILUTION
(HALVING)

Figure 17. Typical titration curves of whole-grain antigens titrated with Cherokee whole-grain antiserum. Initial antigen concentration 1:330

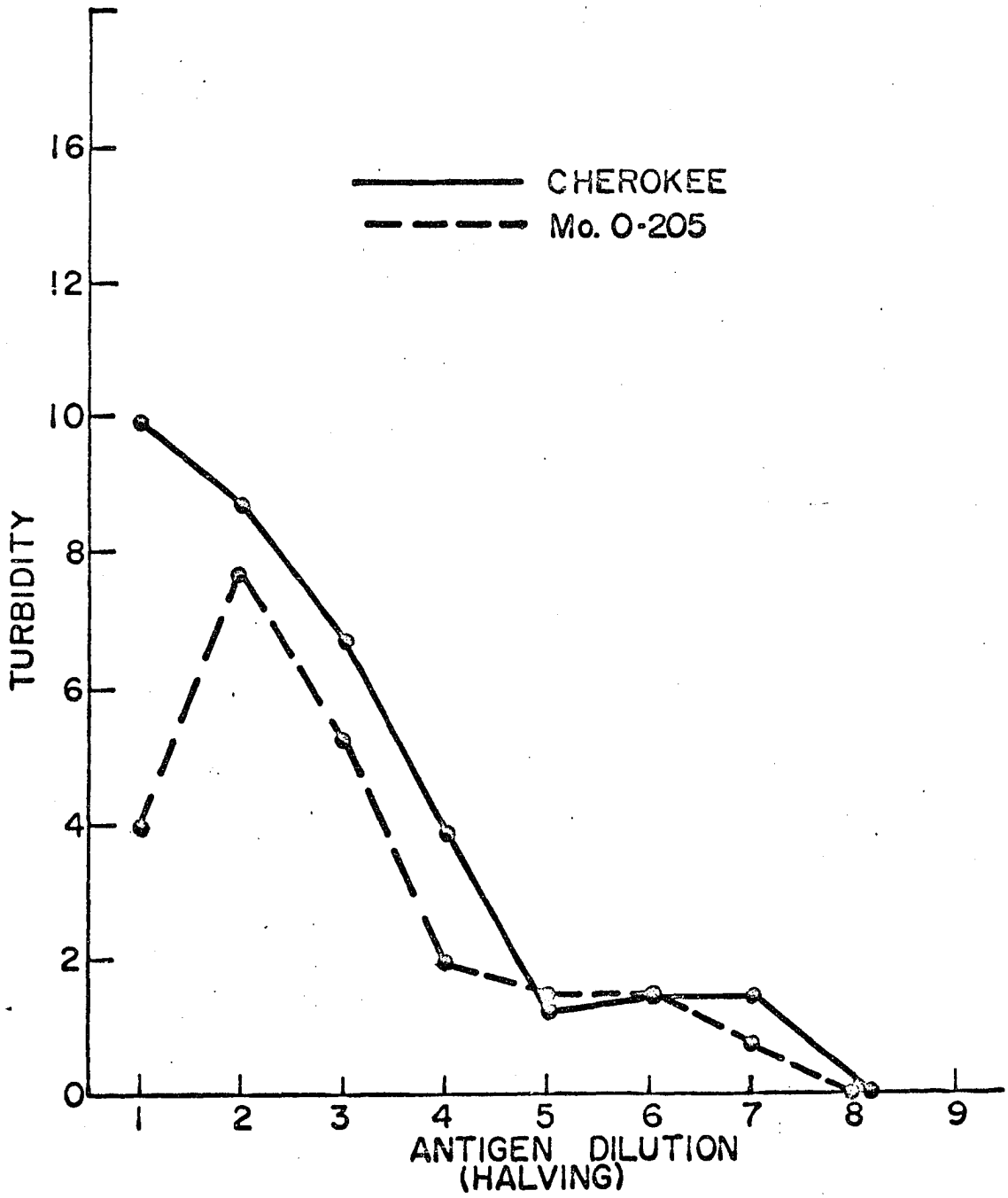

CHEROKEE
Mo. 0-205
TURBIDITY
0
2
4
6
8
10
12
16
1
2
3
4
5
6
7
8
9
ANTIGEN DILUTION
(HALVING)

Figure 18. Typical titration curves of scutellum antigens with Cherokee scutellum antiserum (adjusted for normal serum reactions). Initial antigen concentration 1:500

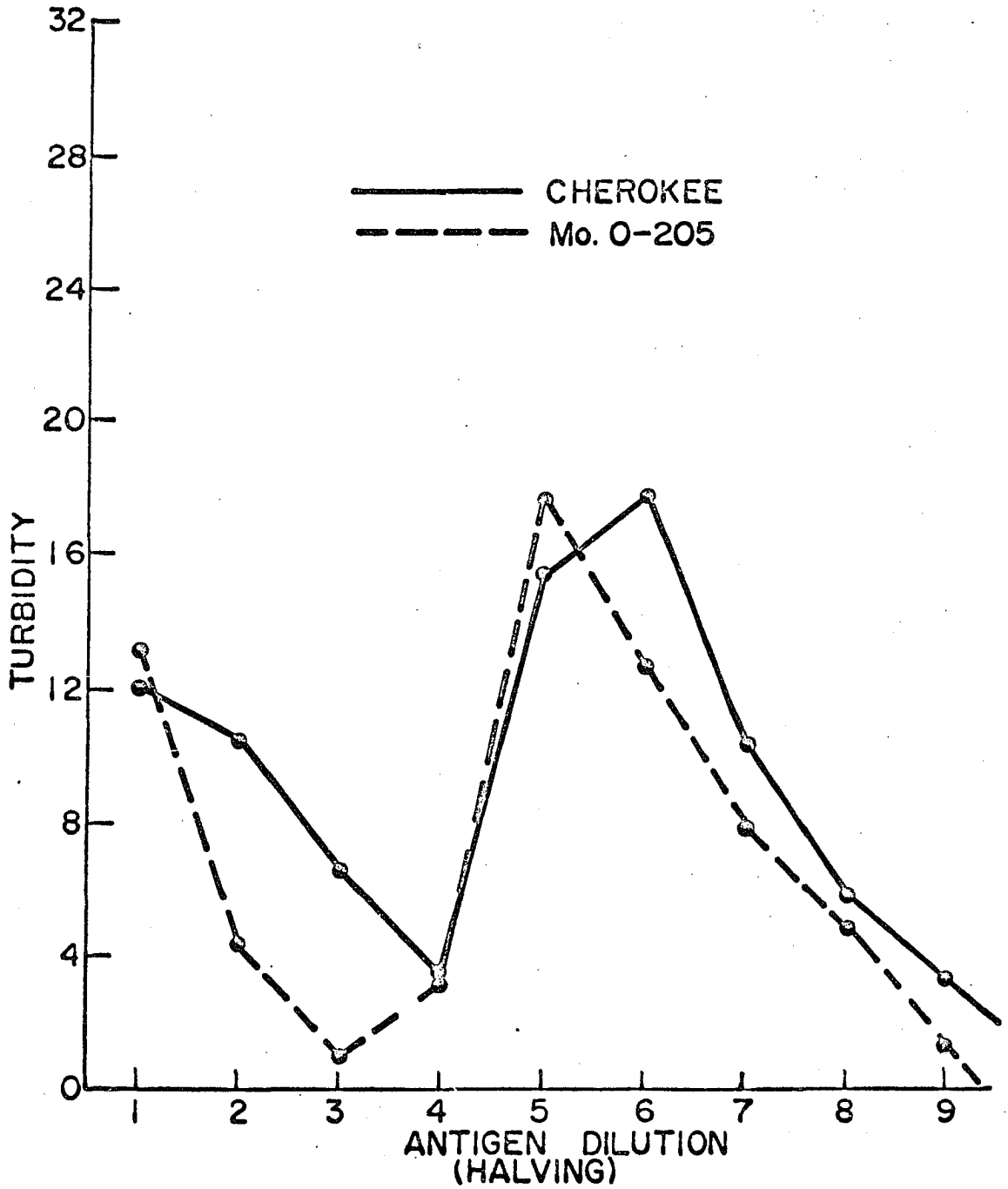

32
28
24
20
16
12
8
4
0
TURBIDITY
CHEROKEE
Mo. 0-205
1
2
3
4
5
6
7
8
9
ANTIGEN DILUTION
(HALVING)

Figure 19. Typical titration curves of embryo antigens titrated with Cherokee embryo antiserum. Initial antigen concentration 1:500

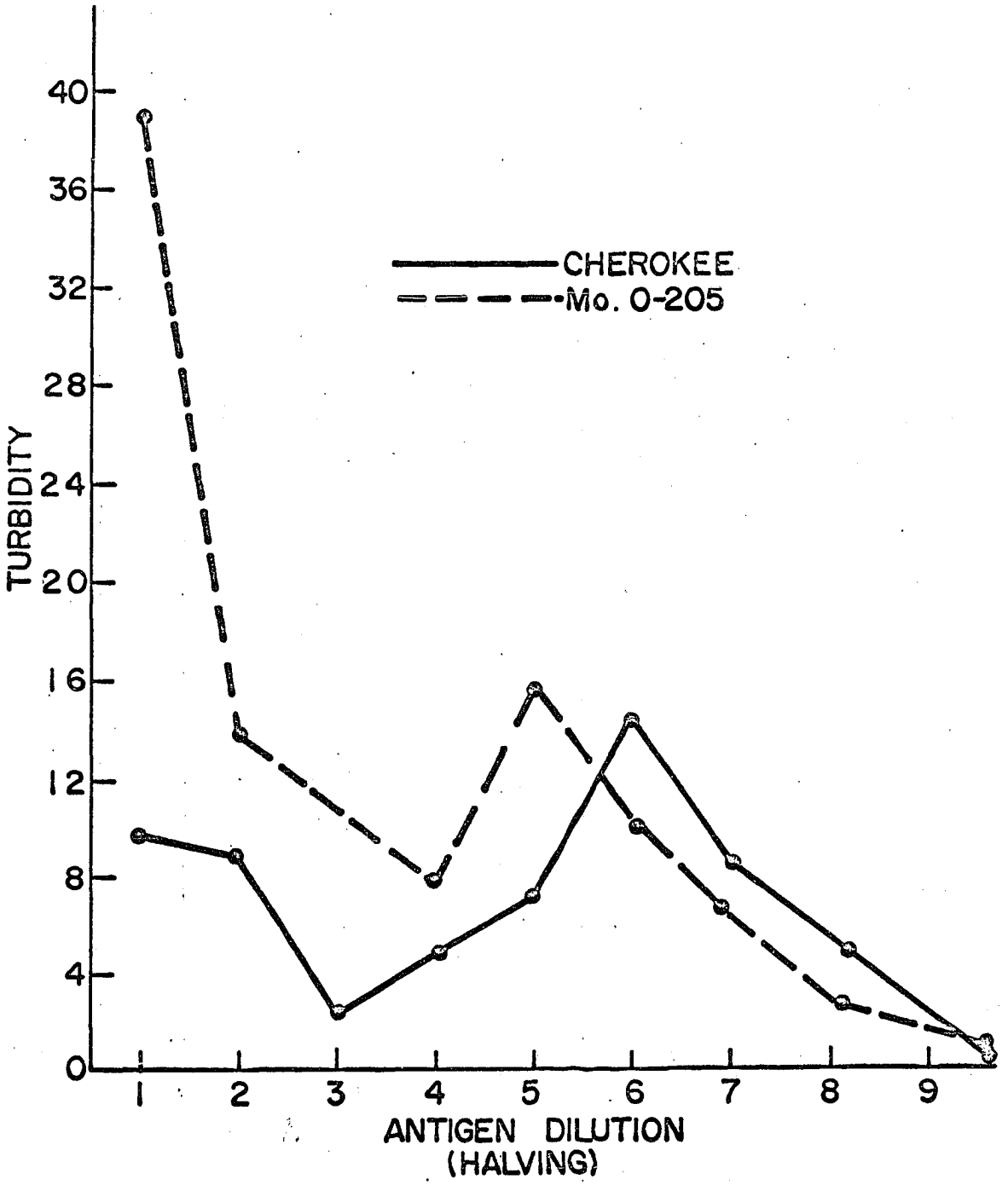
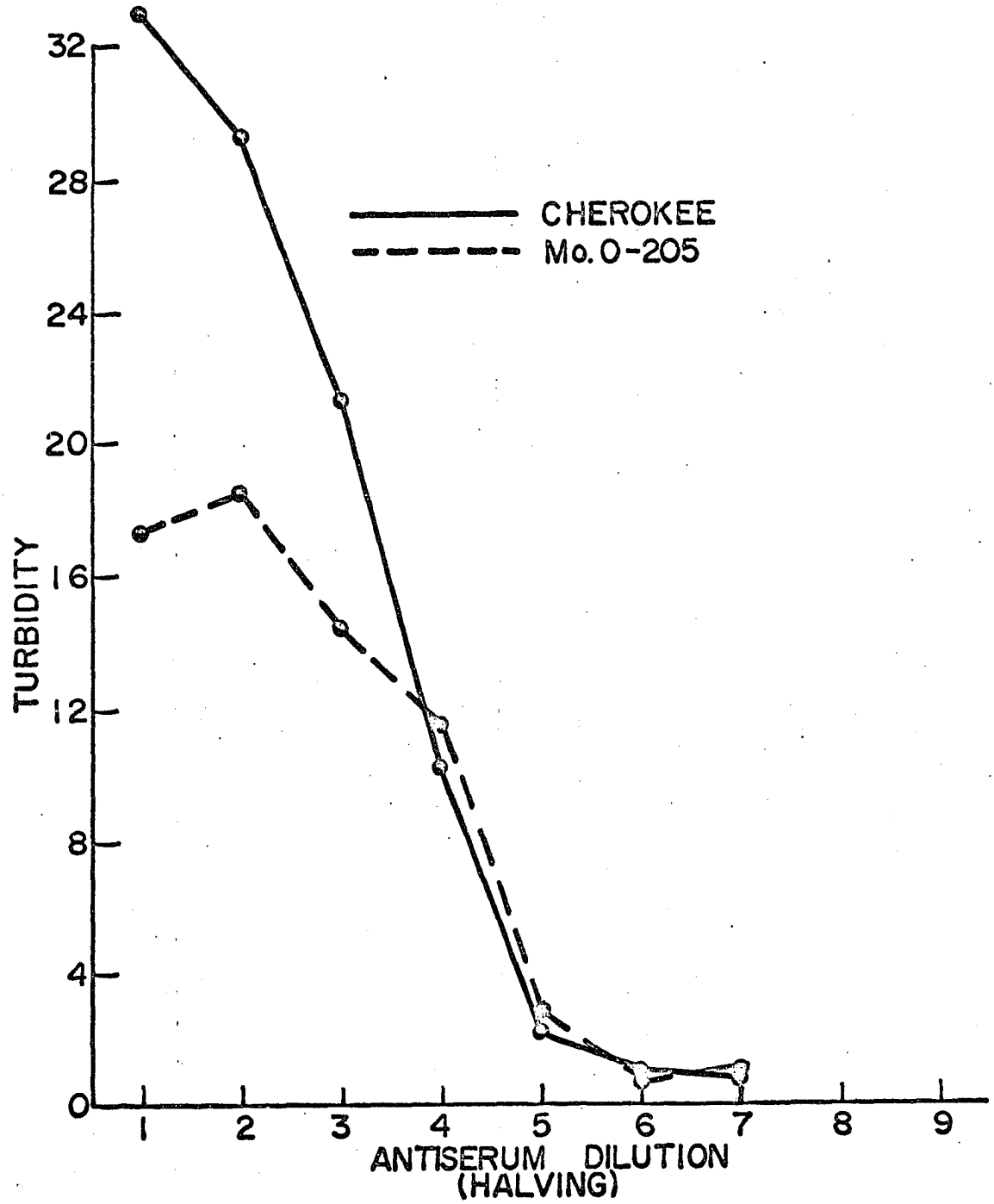

CHEROKEE
Mo. 0-205
TURBIDITY
0
4
8
12
16
20
24
28
32
36
40
1
2
3
4
5
6
7
8
9
ANTIGEN DILUTION
(HALVING)

Figure 20. Typical titration curves of serial dilutions of Cherokee embryo antiserum titrated with constant concentrations (125 ug/ml) of Cherokee and Mo. 0-205 embryo antigens. Initial antiserum dilution 1:2


32
28
24
20
16
12
8
4
0
TURBIDITY
CHEROKEE
Mo. 0-205
1
2
3
4
5
6
7
8
9
ANTISERUM DILUTION
(HALVING)